ROLE OF HER2/neu EXPRESSION IN COLORECTAL CARCINOMAS AND ITS CORRELATION WITH HISTOLOGICAL GRADES

DISSERTATION

SUBMITTED FOR M.D.[PATHOLOGY]

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DECLARATION

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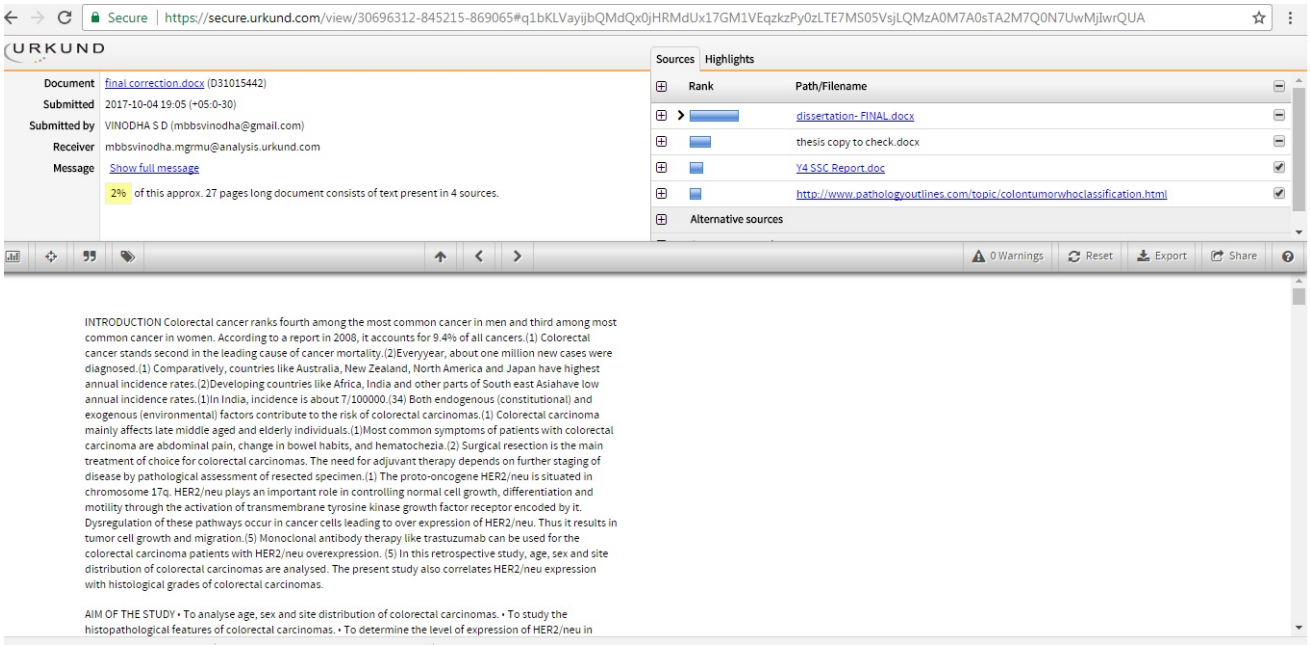

INTRODUCTION Colorectal cancer ranks fourth among the most common cancer in men and third among most common cancer in women. According to a report in 2008, it accounts for 9.4% of all cancers.(1) Colorectal cancer stands second in the leading cause of cancer mortality.(2)Everyyear, about one million new cases were diagnosed.(1) Comparatively, countries like Australia, New Zealand, North America and Japan have highest annual incidence rates.(2)Developing countries like Africa, India and other parts of South east Asiahave low annual incidence rates.(1)In India, incidence is about 7/100000.(34) Both endogenous (constitutional) and exogenous (environmental) factors contribute to the risk of colorectal carcinomas.(1) Colorectal carcinoma mainly affects late middle aged and elderly individuals.(1)Most common symptoms of patients with colorectal carcinoma are abdominal pain, change in bowel habits, and hematochezia.(2) Surgical resection is the main treatment of choice for colorectal carcinomas. The need for adjuvant therapy depends on further staging of disease by pathological assessment of resected specimen.(1) The proto-oncogene HER2/neu is situated in chromosome 17q. HER2/neu plays an important role in controlling normal cell growth, differentiation and motility through the activation of transmembrane tyrosine kinase growth factor receptor encoded by it. Dysregulation of these pathways occur in cancer cells leading to over expression of HER2/neu. Thus it results in tumor cell growth and migration.(5) Monoclonal antibody therapy like trastuzumab can be used for the colorectal carcinoma patients with HER2/neu overexpression. (5) In this retrospective study, age, sex and site distribution of colorectal carcinomas are analysed. The present study also correlates HER2/neu expression with histological grades of colorectal carcinomas.

AIM OF THE STUDY • To analyse age, sex and site distribution of colorectal carcinomas. • To study the histopathological features of colorectal carcinomas. • To determine the level of expression of HER2/neu in

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CONTENTS

ABBREVIATIONS

HER2	-	Human epidermal growth factor receptor 2
HPE	-	Histopathological examination
IHC	-	Immunohistochemistry
FISH	-	Fluorescene in situ hybridisation
WHO	-	World health organization
TNM	-	Tumour node metastasis
FAP	-	Familiam adenomatous polyposis
MSI	-	Microsatelliate instability
AJCC	-	American joint committee on cancer
APC	-	Adenomatous polyposis coli
EGFR	-	Epidermal growth factor
EC	-	Enterochromaffin cell
PP	-	Pancreatic polypeptide
PYY	-	Polypeptide YY

INTRODUCTION

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Colorectal cancer ranks fourth among the most common cancer in men and third among most common cancer in women. According to a report in 2008, it accounts for 9.4% of all cancers.[1] Colorectal cancer stands second in the leading cause of cancer mortality.[2]Every year, about one million new cases were diagnosed.[1]

Comparatively, countries like Australia, New Zealand, North America and Japan have highest annual incidence rates.[2]Developing countries like Africa, India and other parts of South east Asia have low annual incidence rates.[1]In India, incidence is about 7/100000.[34]

Both endogenous (constitutional) and exogenous (environmental) factors contribute to the risk of colorectal carcinomas.[1] Colorectal carcinoma mainly affects late middle aged and elderly individuals.[1]Most common symptoms of patients with colorectal carcinoma are abdominal pain, change in bowel habits, and hematochezia.[2]

Surgical resection is the main treatment of choice for colorectal carcinomas. The need for adjuvant therapy depends on further staging of disease by pathological assessment of resected specimen.[1]

The proto-oncogene HER2/neu is situated in chromosome 17q. HER2/neu plays an important role in controlling normal cell growth,

differentiation and motility through the activation of transmembrane tyrosine kinase growth factor receptor encoded by it. Dysregulation of these pathways occur in cancer cells leading to overexpression of HER2/neu. Thus it results in tumor cell growth and migration.[5]

Monoclonal antibody therapy like trastuzumab can be used for the colorectal carcinoma patients with HER2/neu overexpression.[5]

In this retrospective study, age, sex and site distribution of colorectal carcinomas are analysed. The present study also correlates HER2/neu expression with histological grades of colorectal carcinomas.

AIM OF THE STUDY

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- To analyse age, sex and site distribution of colorectal carcinomas.
- To study the histopathological features of colorectal carcinomas.
- To determine the level of expression of HER2/neu in colorectal carcinomas.
- To correlate the level of HER2/neu expression with various histological grades of colorectal carcinoma.

MATERIALS

&

METHODS

MATERIALS AND METHODS

Fifty cases of colorectal carcinomas were diagnosed in our pathology department, Thanjavur Medical College during period of January 2016 to June 2017.

Study design : Longitudinal retrospective study

Study period : January 2016 to June 2017.

Sample : A total of 50 colectomy specimens received from General surgery and Surgical Gastroenterology department of Thanjavur Medical College.

Inclusion criteria

All cases that are diagnosed as colorectal carcinomas from resected colectomy specimen were included in the study.

Exclusion criteria

1. Colonoscopic biopsies
2. Cases with poor clinical history.

Method of data collection:

All the 50 cases included in the present study were evaluated for the above mentioned criteria.

All information regarding age, sex, clinical presentation and colonoscopic findings were collected.

All hemicolectomy / pancolectomy/ abdominoperineal resection / low anterior resection specimens of colorectal carcinomas were fixed in 10% neutral buffered formalin in toto. Specimen were grossed and macroscopic features are noted. Tissue bits were taken from selected areas of tumours in resected specimens. After routine tissue processing , 4-5 micron thickness sections were taken and stained with routine hematoxylin and eosin stain for histopathological examination. (Appendix 1)

Histopathological examination was done to assess the grade and extent of tumour invasion.

For colorectal carcinoma, staging was done based on TNM staging.

HER2/neu Immunohistochemistry

Among 50 cases of colorectal carcinoma, 25 cases were selected for evaluating the level of HER2/neu expression. Sections of 4micron thickness were taken from paraffin embedded blocks after routine tissue processing. Using advanced polymer staining system, these sections of colorectal carcinomas were stained with antibody directed against HER2/neu.

Immunohistochemical staining was done according to protocol (Appendix 2). These Immunohistochemical slides were assessed for percentage

of tumor cells that have stained membranous positivity and also the intensity of staining is noted.

Method of scoring (6,7)

0 - No staining at all or membrane staining in <10% of tumour cells.

1+ - Faint / barely perceptible membrane staining in >10% of tumour cells.

2+ - Weak to moderate staining of entire membrane in >10% of tumour cells.

3+ - Strong staining of entire membrane in >10% of tumour cells.

Staining score of 2+ and 3 + considered as positive [6,7].

HER2/neu expression in colorectal carcinomas were correlated with its histological grades and statistical analysis was performed based on it. Statistical analysis was done using Fishers exact test. p value less than 0.05 ($p<0.05$) was taken to indicate statistical significance.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Colorectal cancer is a major world wide health problem [2]. Based on estimation given by GLOBOCAN Project in 2008, about 1,235,108 colorectal cancer cases were registered worldwide and it accounts about 609, 051 deaths globally[5]. Its worldwide incidence rate is about 2% annually [8]. In 2009, according to U.S.Surveillance, Epidemiology and end results (SEER) program statistics, colonic carcinoma had an incidence of 30.45 per 100,000 and rectal carcinoma had an incidence of 12.16 per 100,000[1]. In United States, lifetime risk for developing colorectal carcinoma in both males and females estimated to be 6% [2]. From 1990 to 2012, incidence of colorectal tumours increases by about 200,000 new cases per year.[9]

Anatomy of Large Intestine [3]

The extend of large intestine is from ileocaecal junction to anus. It measures about 1.5 metre long. The parts of large intestine are caecum, ascending colon, transverse colon, descending colon, sigmoid colon,rectum and anal canal. About 2 cm below the ileocaecal orifice , worm - like diverticulum called vermiform appendix arises from the posteromedial wall of the caecum.

Histology

The large intestine is made up of four layers

1) Mucosa

Mucosa is further divided into epithelium, lamina propria and muscularis mucosa.

2) Submucosa

3)Muscularis propria

4) Serosa

The surface epithelium is lined by columnar cells into which the crypts open. In addition,it also contains goblet cells, endocrine cells and occasional paneth cells. Follicle associated epithelial cells or ‘M’ cells (M for microfold or membrane cells) are cells that lie in the epithelium overlying the lymphoid follicles of lamina propria.

Columnar cells are absorptive with striated border. These cells play an important role in the absorption of water and electrolytes from intestinal contents.

The main function of goblet cells is synthesis and secretion of mucin granules. Paneth cells may be seen occasionally in caecum, proximal right colon. Its major function is to secrete lysozyme and epidermal growth factor.

Lamina propria contains tubular glands which is formed by deep crypt epithelium. These glands are surrounded by lymphoid follicles. Submucosa contains loose connective tissue and submucosal plexus of Meissner.

Muscularis propria consists of inner circular layer and outer longitudinal layer of smooth muscle. Myenteric plexus of Auerbach lies between these two muscle layers. Outer longitudinal layer is collected to form three thick bands called Taenia coli. In rectum, taenia coli is absent and it contains a complete longitudinal muscle coat.

Serosa is absent over posterior aspect of ascending and descending colon.Also most of the rectum is devoid of serosa except front and sides of upper one-third and front of middle third.

Etiology [1,2,9]

- Both environmental and genetic factors play an important role.
- Modifiable risk factors for colorectal carcinoma includes obesity, red meat, smoking, alcohol. Other risk factors are inflammatory bowel disease, oral contraceptive use, high caloric intake.
- Obesity is one of the risk factor for colorectal carcinoma. This may be due to increased serum insulin production which inturn causes cellular proliferation in intestine.

- Protective factors for colorectal carcinoma includes high calcium intake, folate intake, selenium intake, dietary fiber intake.
- According to Nurses Health study report, women who consumed redmeat have 2.5 fold increase risk for developing colorectal cancers than the women who do not consume.
- High fat diet results in bile acid production which inturn stimulates colonic epithelial proliferation thus resulting in tumour production.
- Dietary Fibre plays a protective role by luminal dilution and binding of toxic substances, inhibition of carcinogens due to decreased colonic pH, less mucosal contact by fecal mutagens by decreasing colonic transit time.
- Short chain fatty acids are produced by fermentation of dietary fibres by anaerobic organisms. These fatty acids increases fecal acidity thereby inhibiting carcinogen and bile acid metabolism.
- There is a risk of colorectal carcinoma in patients who undergone cholecystectomy and gastrectomy, probably due to increased bile acids which inturn leads to colonic epithelial proliferation.
- Vitamin A, Beta - carotene and vitamin E are protective due to its antioxidant properties.
- According to a study, vitamin D and calcium intake proved to be protective against colorectal carcinoma. These agents form insoluble

calcium soap in combination with bile acids or fatty acids, thus mucosal damage was prevented.

- The frequency of rectal carcinoma was more frequent in persons with heavy beer drinking.
- If there is a family history of colorectal carcinoma, other family members had 21% life time risk for developing colorectal cancer.
- Genetic syndrome that predispose to colorectal carcinoma include familial adenomatous polyposis, juvenile polyposis syndrome, cowden syndrome, serrated polyposis, heriditary polyposis colorectal cancer etc.
- Colorectal carcinoma due to genetic factors constitutes about 20%.

Pathogenesis [4]

There is heterogenous groups of molecular events behind the pathogenesis of colonic carcinoma which includes genetic and epigenetic abnormalities. Two genetic pathways mainly associated are APC/ß - catenin pathway and microsatellite instability pathway.

About 80% of colonic tumours (sporadic) are due to classic adenoma - carcinoma sequence. This is caused by APC mutation in the earlier stage of neoplastic process. Mutation or epigenetic events causes functional inactivation of two copies of APC gene resulting in development of adenomas. APC negatively regulates ß - catenin by binding and promoting its degradation. If APC function is lost, accumulation of ß- catenin results. This inturn leads to formation of DNA-binding factor TCF complex and activation of gene transcription (MYC and Cyclin D1). These molecular events finally results in proliferation.

In many colonic tumours, instead of APC mutations, ß-catenin mutations results in same impact by preventing degradation caused by APC. In addition to these mutations, KRAS mutation also causes tumour growth by preventing apoptosis. Mutations in genes encoding SMAD2 and SMAD4 (tumour suppressor genes) also results in neoplastic growth since they are associated with TGF-ß signaling. Normal function of TGF-ß is cell cycle inhibition. Thus mutation of these genes leads to uncontrolled cell growth.

TP53 is a tumour suppressor gene. Its mutation was reported in about 80% colonic tumours. In adenomas, TP53 mutations are uncommon. This suggests that TP53 mutations can occur at later stages of carcinogenesis.

The main hallmark behind APC/ß-catenin pathway was chromosomal instability.

Microsatellite instability refers to accumulation of mutations in microsatellite repeats in patients having DNA mismatch repair deficiency. They are known as MSI high (MSI -H) tumours. Cell growth is regulated by certain microsatellite sequences which are situated in coding or promoter regions of genes eg.genes encoding type II TGF-ß receptor and BAX protein. The function of TGF-ß is to inhibit cell growth so its receptor mutation results in unrestrained growth. Loss of proapoptotic protein BAX increase cell proliferation due to apoptotic inhibition.

CPG island hypermethylation phenotype (CIMP) was demonstrated in colonic tumours with microsatellite instability. Many of these tumours harbor hypermethylation of promoter region MLH 1 and reduced repair function.

BRAF mutations are frequently seen in these tumours. Thus, signature of microsatellite instability pathway of carcinogenesis was microsatellite instability, mutation in BRAF and MLH 1 methylation.

Colonic tumours with TP53 mutations lack CPG island methylator phenotype. Tumours with microsatellite instability and CPG island hypermethylation phenotype are commonly situated in proximal colon.

COLORECTAL CARCINOMA

WHO CLASSIFICATION OF TUMOURS OF COLON AND RECTUM

Epithelial tumours	**Types**
Adenoma	Tubular villous Tubulovillous Serrated
Intraepithelial neoplasia (dysplasia)	Low grade glandular intraepithelial neoplasia High grade glandular intraepithelial neoplasia
Carcinoma	Adenocarcinoma Mucinous adeno carcinoma Signet ring cell carcinoma Small cell carcinoma Squamous cell carcinoma Adenosquamous carcinoma Medullary carcinoma Undifferentiated carcinoma
Carcinoid (well differentiated endocrine neoplasm)	EC-cell, Serotonin - producing neoplasm L-cell, Glucagon - like peptide and PP/PYY producing tumour Others
Mixed carcinoid-adeno carcinoma	
Others	

Non - Epithelial Tumours

1. Lipoma
2. Leiomyoma
3. Gastrointestinal stromal tumour
4. Leiomyosarcoma
5. Angiosarcoma
6. Kaposi Sarcoma
7. Malignant melanoma
8. Others

Malignant lymphomas

- Marginal zone lymphoma
- Mantle cell lymphoma
- Diffuse large B cell lymphoma
- Burkitt lymphoma
- Others

Secondary Tumours

Adenocarcinoma [1,10,11]

Adenocarcinoma constitutes about 85% of colorectal carcinomas. Among them, moderately to well differentiated are common. Prominent villous or papillary component can be seen in some tumours at the surface. It may penetrate all the bowel layers into pericolic fat and perineural spaces. Venous invasion can also be seen.

Based on degree of gland formation, it is divided into three grades. Grade I tumours constitutes about 15 to 20% of colorectal adenocarcinomas, grade II tumours constitutes about 60 to 70% and grade III tumours constitutes about 15 to 20% of colorectal adenocarcinomas. Thus, grade I and II tumours are low grade tumours whereas grade III and undifferentiated carcinoma are high grade tumours. Colorectal carcinomas are termed as grade III if more than 50% of tumour composed of poorly differentiated component.

If the tumour cells are detached and migrated into the stroma at the advancing edge of the tumour, it is termed as tumour budding. Histologically, tumour buds should composed of five tumour cells or less at the advancing edge of the tumour.

Mostly, grade I and II tumours are associated with greater degree of tumour budding. Tumour budding was associated with poorer prognosis.

Mucinous adenocarcinoma[12]

If tumour composed of more than 50% of mucin with tumour cells floating in pools of extracellular mucin, it is termed as mucinous adenocarcinoma.About 15% of colorectal carcinoma are mucinous carcinomas and its most common location is rectum. Most of the microsatellite instability (MSI - H) carcinomas belong to this type. Mucinous carcinomas have higher incidence of extensive perirectal spread and involvement of lymph nodes than non-mucinous colorectal tumours. Prognosis is poor compared to conventional adenocarcinoma.

Signet ring cell carcinoma[13]

It is also known as Linitis plastica type carcinoma. If a tumour contains more than 50% tumour cells with prominent intracytoplasmic mucin, it is termed as signet ring cell carcinoma. Grossly, it usually present as diffuse infiltration of wall. Young patients are commonly affected. MSI-H carcinomas constitutes about one third of cases. It is an aggressive tumour with bad prognosis.

Small cell carcinoma[14]

Its histological features is similar to small cell carcinoma of lung. Commonest site is right colon. It has poor prognosis with lymph nodes and liver metastases at earlier stage. They express positivity for neuron specific enolase, synaptophysin, chromogranin and Leu-7.

Squamous and adenosquamous carcinomas[15]

These are very rare tumours. They are commonly seen in patients with ulcerative colitis, schistosomiasis and pelvic irradiation.

The criteria that is essential to make the diagnosis of squamous or adenosquamous carcinoma of colorectum are

1. There must be no other sites of squamous cancer in the body.

2. There must be no involvement of cloacogenic or squamous lined mucosa.

Medullary carcinoma[16]

Rare tumour among colorectal tumours. It is composed of neoplastic cells arranged in sheets with prominent lymphocytic infiltration. Medullary carcinomas are commonly situated in proximal colon with female preponderance. These tumours have good prognosis and lymph node metastasis is less compared to other tumours. Prognosis is better than poorly differentiated colorectal carcinomas.

Undifferentiated Carcinoma[17]

Undifferentiated carcinomas are malignant epithelial tumours without any differentiation. They are different from poorly differentiated carcinomas since the later contains intracytoplasmic mucin.

Carcinoids and Neuroendocrine Carcinoma[18]

These tumours are rare among colorectal carcinomas. They usually occurs in caecum and rectum. Conventional adenocarcinomas can show focal neuroendocrine differentiation.

Serrated Adenocarcinoma[11]

Serrated adenocarcinoma was a recently proposed entity. It comprises 7.5% of all colorectal tumours. It accounts for about 10-15% of proximal colonic carcinomas with female preponderance. Serrated adenocarcinoma associated with MSI-L or MSS had worse prognosis than those that are associated with MSI-H.

Histological features of serrated adenocarcinomas are

1. Epithelium has a serrated pattern with no loss of polarity. Cells have abundant eosinophilic cytoplasm with bland or vesicular nuclei.
2. Mucinous differentiation was found in 43% of these tumours. Histologically, these tumours composed of mucin pools containing eosinophilic papillary rods and cell balls. They are different from classical mucinous carcinomas.
3. Trabecular pattern was seen in poorly differentiated serrated adenocarcinomas.

Micropapillary adenocarcinoma[11]

This is an aggressive tumour. Histologically, micropapillary adenocarcinoma composed of neoplastic cells in balls or clusters separated by cleft like spaces. The diagnosis of micropapillary adenocarcinoma requires the presence of atleast 5% tumour cells with micropapillary features. Lymph node metastasis is more frequent.

Carcinoid tumour

Grossly, it may present as depressed plaque or polypoid lesion. EC-cell tumours are serotonin producing with histological features similar to its counterpart in jejunoileal region. L-cell tumours are glucagon like peptide and PP/PYY - producing tumours. L-cell tumours composed of predominant ribbon

pattern (Type B) with admixture of tubular, acinar or irregular trabeculae with rosettes (Type C) and sometimes, areas of solid nests (Type A). In EC-cell tumours, type A structures are seen.

Mesenchymal tumours

Mesenchymal tumours in large intestine are very rare. Common sites of lipoma are caecum and sigmoid colon.

Gastrointestinal stromal tumour (GIST) affects individuals between 6th and 8th decades. Malignant GIST are common in colorectum than benign ones.

Leiomyomas usually present as small polyps in colorectal region arising from muscularis mucosae. Vascular tumours like hemangiomas, lymphangiomas, hemangioendothelioma and angiosarcoma can be seen in colorectal region.

Malignant Lymphoma

Frequency of lymphomas is less in large intestine.It accounts for 0.2% of colorectal tumours. Mostly older patients are affected. Common sites of malignant lymphoma in colorectal region are distal large intestine and rectum. Mucosa Associated Lymphoid Tissue type (MALT) is common lymphoma seen in colon.

Diffuse large B-cell lymphoma and Burkitt lymphoma usually present as bulky masses with stricture and ulceration. Mantle cell lymphoma tend to form isolated mass or present as multiple polyps. MALT lymphomas may remain asymptomatic for prolonged periods but loco-regional lymph nodes are frequently involved. Among these lymphomas, mantle cell lymphoma has an aggressive behavior since it presents at advanced stage with metastasis in spleen, mesenteric and peripheral lymph nodes. Often, bone marrow and peripheral blood involvement can also be made out.

Grading of Colorectal adenocarcinoma[(1)]

Grade	**Descriptive nomenclature**	**Criteria**	**AJCC recommendation**
G_x	Grade can not be assessed	-	-
G_1	Well differentiated	95% gland forming	Low grade
G_2	Moderately differentiated	50% - 95% gland forming	Low grade
G_3	Poorly differentiated	<50% gland forming	High grade
G_4	Undifferentiated	No apparent gland formation	High grade

Staging of colorectal carcinoma

In colorectal carcinomas, tumour behaviour and outcome is mainly determined by anatomic extent of tumour spread. Pathologic evaluation helps in clinical management of patients and research purposes. It also helps in documentation of lesion in case of follow up studies.

DUKE'S staging system [11]

In 1932, Dukes explained a staging system which is less complicated

A	Tumour confined to the intestinal wall
B	Tumour invading through the intestinal wall.
C	With lymph node(s) involvement
C_1	Only the regional lymph nodes are involved
C_2	Nodes at the point of mesenteric blood vessel ligature are involved
D	Distant metastasis

ASTLER - COLLER STAGING SYSTEM [11]

A	Tumour limited to the mucosa
B_1	Tumour involving muscularis externa but not penetrating it.
B_2	Tumour penetrating through muscularis externa

C_1	Tumour confined to the bowel wall with regional lymph node metastases
C_2	Tumour penetrating through the wall with regional lymph node metastases
D	Distant Metastases

Additional Staging

Venous invasion (v)

V_0 - No venous invasion

V_1 - Microscopic venous invasion

V_2 - Macroscopic venous invasion

Lymphatic invasion (L)

L_0 - No lymphatic vessel invasion

L_1 - Lymphatic vessel invasion

TNM staging system was developed by the American Joint Communication on Cancer (AJCC). This staging system is based on

T - Degree of tumour invasion

N - Lymph node involvement status

M - Distant metastasis

AJCC	TNM STAGE	CRITERIA [19, 20]
Stage 0	Tis N_0M_0	Tis : Tumour confined to mucosa; cancer - in-situ
Stage I	$T_1N_0M_0$	T_1 : Tumour invades submucosa
	$T_2N_0M_0$	T_2 : Tumour invades muscularis propria
Stage II A	$T_3N_0M_0$	T_3 : Tumour invades subserosa or pericolic tissue
Stage II B	$T_4N_0M_0$	T_4 : Tumour directly invades other organs or structures and /or perforates visceral peritoneum
Stage III A	$T_{1-2}N_1M_0$	N_1 : Metastasis to 1-3 regional lymph nodes
Stage III B	$T_{3-4}N_1M_0$	
Stage III C	Any TN_2M_0	N_2 : Metastasis to 4 or more regional lymph nodes
Stage IV	Any T AnyN M_1	M_1 : Distant metastasis present

HER2/neu

HER2/neu also called as ErbB-2, c-erbB2, HER-2. It belongs to the family of Human epidermal growth factor receptor (HER). There are many members of Human epidermal growth factor receptor like HER-1 (EGFR or ErbB1), HER-2, HER-3 (ErbB3) and HER-4 (ErbB4). 185 - KDa plasma membrane-bound tyrosine kinase receptor is situated on chromosome 17q21. This tyrosine kinase receptor is encoded by HER-2, which is a proto oncogene. Phosphoinositide-3-kinase, mitogen activated protein kinase (MAPK), phospholipase C and protein kinase C are activated when HER2/neu is stimulated by extracellular signals.

Thus it results in signal transduction and transcription [27]. Thus it controls cellular proliferation and differentiation. It also inhibits apoptosis and tumour growth. It plays an important role in cell to cell and cell to stromal communication. Two copies of HER-2 gene present in all normal epithelial cells. HER2 receptors are expressed in low levels on the surface of cells. Carcinomas of breast, ovary, colon, uterus, stomach, prostate and lung shows HER2/neu overexpression and its amplification. The expression of HER2/neu in tumours of colorectal region varies from 0% to 83%.[6]

There are many methods to assess HER2/neu expression. Immunohistochemistry is commonly used.[23]

A Pappas et al [5] in his study, evaluated HER2/neu expression in 51 colorectal carcinomas using Immunohistochemical technique. Among them,22 cases were colonic carcinomas and 29 cases were rectal carcinomas. They found that 3.9% of colorectal carcinoma cases showed HER2/neu overexpression. According to this study, most of the tumours are grade 2 (84.3%) when compared to grade 1 and grade 3 tumours. No correlation was found between patient's age, sex and clinicopathological details. The factors influencing HER2/neu expression are tissue fixation,primary antibody choice, antigen retrieval process, antigen dilution and time given for antigen retrieval and peroxidase reaction. There is disagreement between membranous and

cytoplasmic staining since cytoplasmic staining of HER2/neu is noticed in more number of cases. This study considered only membranous positivity.

Manmeet Kaur Gill et al[8] performed a study in 20 colorectal carcinoma cases. In their study, metastasis was reported in 10 nodes (50%) and HER2 expression was noticed in all ten nodes positive for metastasis. Out of ten remaining reactive nodes, HER2 expression was noticed in four cases. There was an association between HER2 staining and lymph nodes metastasis with a statistically significant p value 0.015. The difference in HER2/neu expression may be due to variation in tissue fixation, different patterns of HER2 scoring and varying IHC procedures.

Sadia Anwar et al [6] in his study of 100 colorectal tumours, observed that 42% cases showed HER2 positivity. Among them, moderate membranous positivity (2+) was noticed in 28.5% (n=12) of conventional adenocarcinomas and 30.7% (n=8) mucinous carcinomas. Strong membranous positivity (3+) was observed in 52.3% (n=22) of conventional adenocarcinomas and 42.8% (n=6) of mucinous carcinoma cases. Signet ring cell carcinomas showed negative staining. There was an inverse association between HER2/neu score and histological grade with a significant statistical value p=0.001. Thus maximum HER2/neu overexpression noticed in low grade tumours than high grade tumours. There was no association between tumour histology, grade, age, sex, location and clinical presentation ($p>0.05$).

According to Essapen et al [51], 41% cases out of 170 cases of colorectal tumours showed strong positivity for HER2/neu immunohistochemistry.

An Na Seo et al [23] reported that (in cohort 1) out of 365 patients, 14 cases (3.8%) had moderate membranous staining (2+) and 8 cases (2.2%) had strong membranous staining (3+) for HER2/neu. In cohort 2, out of 174 advanced colorectal carcinoma patients, 5 cases (2.9%) had moderate membranous positivity (2+) and 5 cases (2.9%) showed strong membranous positivity (3+) for HER2/neu IHC. In cohort 1, 2.5% cases and in cohort 2, 2.3% cases was detected with HER2 mRNA overtranscription.There was no correlation between HER2 expression with tumour infiltration, lymph node status, distant metastasis and perineural invasion ($p>0.05$). There was correlation between HER2 expression and tumour site ($p=0.033$) in cohort 1. There was no association between HER2 mRNA overtranscription with clinicopathological details except tumour site ($p=0.001$ in cohort 1, $p=0.026$ in cohort 2). There was correlation between HER2 gene amplification and tumour site.

Soo Kyung Nam et al[21] performed a study on 191 colorectal carcinoma patients in advanced stage with distant metastasis. They observed wild type KRAS mutation in 87 (45.5%) cases and KRAS mutation in 104 (54.5%) cases. Among them, mutation in codon 12 or 13 was noticed in 97 (93.3%) cases and codon 61 mutation noticed in 7 (6.7%) cases. 3.1% tumours was detected with

BRAF mutations. PIK3CA mutations was detected in 13.1% (n=25). HER2 gene amplification was observed in 8.4% (n=16) tumours by SISH analysis. Among 191 cases, 1.6% were MSI-H and 98.4% tumours were MSS/MSI-L. There was an association between PIK3CA mutations and KRAS mutations (p=0.020). HER2 amplification and mutation in BRAF were commonly noticed in KRAS wild type rather than mutant type. There was no correlation between MSI status and genetic alterations. Proximal colon was commonly affected by KRAS mutant tumours especially low grade tumours whereas distal colon was frequently involved by tumours with HER2 amplifications. There was no correlation between PIK3CA mutations with MSI status or clinicopathologic variables.

Nathanson et al [30] studied HER2/neu expression in 139 cases of colorectal tumours and among them, 5 cases (3.6%) had HER2/neu over expression and 4 cases (2.4%) showed HER2/neu gene amplification. There is no correlation between HER2/neu overexpression and gene amplification with clinicopathologic features or survival rate of patients.

Kavanagh et al [31] performed a study which included 132 colorectal cancer patients and observed that 9 cases (85%) expressed moderate membranous staining and 2 cases (2%) showed strongly positive staining for HER 2/neu immunohistochemistry. There was no association between sex, age, stage, grade, survival rate and recurrence.

A study by park et al [32] in 137 colorectal carcinoma patients showed 65 cases (47.4%) with HER2/neu overexpression. Neither tumour grade nor staging were correlated with each other. They found that tumours with HER2/neu overexpression had increased recurrence rate.

According to Osako et al [33] in his study involving 146 colorectal cancer cases, 100 (68.5%) showed cytoplasmic staining and only 3 cases revealed membranous positivity for HER2/neu.

According to a study by Mckay et al[44], 81.8% tumours expressed HER2/neu. They studied HER2 expression in both primary as well as in metastatic tumours in lymph node. They finally concluded that there was no association between HER2 expression and lymph node metastases.

According to Cancer Genome Atlas(TCGA) Colorectal cancer project, 7% cases had HER2 alterations, 5 cases had HER2 gene amplification. HER-2 mutations and amplification were present in 3 cases.[25]

A study by Schuell et al[7] in 77 colorectal carcinoma cases showed HER2/neu positivity in 30% of cases, with moderate membranous positivity in 1% case and strong positivity in 3% cases, 26% cases with mild membranous positivity. There was no correlation between HER2/neu expression with survival rate and clinicopathological information.

According to Fanotto et al[27] ,HER2 amplification was found in distal carcinomas of colon more frequently when compared to proximal colonic carcinomas.

Won - Suk Lee et al [29] studied HER2/neu expression and KRAS status in 94 colorectal carcinoma patients both in primary tumours as well as in metastatic lesions (lung or liver metastasis). Among them, 2.1% of cases showed strong membranous positivity by IHC whereas 10.1% cases showed expression in HER2 amplification(FISH). In one of these patients, HER2 staining showed positivity in metastatic lesion but not in primary tumour. 64 cases (68.1%) showed negativity for both HER2/neu Immunohistochemistry and FISH; Two cases showed positivity in both IHC and FISH and 7 cases (7.4%) showed equivocal results. 85.1% cases expressed HER2/neu protein expression in both primary and metastatic tumours. Two cases showed positive IHC staining in primary tumour but not in metastasis. There was no correlation between HER2 status and KRAS status (p=0.486). They observed that 87.2% of cases had KRAS mutations in both primary as well as in metastatic lesions. HER2/neu over expression was noticed in 5.3% (n=10) cases with KRAS mutations.

SD Richman et al [28] observed strong HER2 expression in 25 colorectal carcinoma patients with stage II - III tumours and 29 colorectal carcinoma patients with stage IV tumours. HER2 amplification by FISH was seen in 20

colorectal carcinoma patients with stage II - III disease and 27 colorectal carcinoma patients with stage IV disease.There was correlation between HER2/neu expression and KRAS/BRAF WT tumours especially in tumours of stage IV. They proposed that MEK-AKT pathway was activated by HER2 amplification in colorectal carcinoma. In RAS/RAF WT patients, HER2 expression should be assessed to detect the response to the HER2 targeted therapy.

JinhuaTu et al [(26)] evaluated HER2/neu overexpression in 878 colorectal carcinoma specimens and found that out of 878 patients, 102 cases (11.6%) showed HER2 overexpression. Among them, strong positivity (3+) was observed in 25 cases and moderate membranous positivity (2+) was found in 77 patients. According to this study, patients with stage 0, stage I and stage II disease showed more HER2 expression than stage III and stage IV patients. There was no correlation between HER2 overexpression and sex, age, tumour location, staging and lymph node status. Out of 102 cases with strong HER2 membranous positivity, 25 cases (24.5%) showed positivity in HER2 gene amplification. Out of 77 cases with moderate HER2 positivity, only 5 cases(6.5%) expressed positivity in HER2 gene amplification.

Antonacopoulou et al [(35)] in their study of 124 colorectal cancer patients found that 24.7% cases showed HER2/neu overexpression. They also evaluated EGFR and COX-2 levels by IHC. In 46 cases of colorectal cancers, reverse

transcriptase PCR was used to assess mRNA levels. There was association between EGFR expression and mRNA ($p<0.001$). Also HER2 expression ($p<0.004$) and COX-2 expression ($p<0.007$) correlated with mRNA levels. There was no association between EGFR with tumour stage or grade.

Demirbas et al [36] observed HER2 expression in 44.3% of colorectal tumours by IHC. Among these cases, most of the tumours are situated in rectum and they are above 5cm size. HER2/neu overexpression correlates with grade and vascular invasion. Tumours with lymphatic invasion showed more HER2 expression but it was not statistically significant. Tumours of Astler-Coller stage C1 and C2 were frequently observed with HER2 and TP53 overexpression. They demonstrated overexpression of HER2 and TP53 among moderately differentiated and poorly differentiated carcinomas. According to this study, there was an association between HER2 expression in colorectal carcinoma and its prognosis.

According to a study by Kruszewski et al [37], out of 202 colorectal carcinoma patients, 27% cases showed HER2/neu positivity. Among them, 26.7% cases had membranous positivity and 66.3% cases had cytoplasmic positivity. Moderate positivity(2+) was observed in 32% cases and strong positivity(3+) was observed in 15% cases. No association was observed between HER2 expression and other variables like age, sex, site, tumour grade, histological variant, tumour stage.

Kim et al [38] in his study found that HER2/neu was overexpressed in 0.5% of cases out of 185 patients with colorectal carcinoma. They also studied

the expression of COX-2 and Ki-67 protein. 94.3% of cases expressed COX-2 protein and 5.9% cases were found with Ki-67 expression. No correlation was found between HER2, COX-2, and Ki-67 expression.

Marx et al [39] studied HER-2 expression using tissue microarray (TMA) and found that out of 1851 patients, 2.7% patients showed overexpression of HER2/neu. There was no relationship among HER2 amplification with tumour site, grade, tumour histology and stage.

B Ingold Heppner et al [24] reported that among 1645 colorectal cancer cases, 53 cases expressed 1+ HER2/neu staining, 9 cases expressed 3+ strong positivity and 35 cases with equivocal result. They also evaluated HER2/neu status in lymph node metastases of these patients. They found (n=12) similar HER2/neu profile like that of their primary tumour. HER2/neu amplification was detected in 57.8% (n=26) cases. Among them, nine cases had strong positivity and 17 cases had moderate membranous positivity for HER2/neu IHC. There was an significant association between HER2 status and lymph node metastasis. There was no significant correlation between HER2 expression and clinicopathological variables.

Kafi SG et al [40] studied 69 cases with colonic adenocarcinoma and evaluated HER2/neu expression in them to find out the frequency and intensity of HER2/neu staining. Out of 69 cases, 34.1% cases showed membranous

cytoplasmic staining. They observed that there was no association between HER2/neu expression with gender, location and variant of tumour.

Tavangar et al [41] studied 55 colorectal carcinoma patients and performed HER2/neu IHC. Significant correlation found between HER 2/neu over expression and advanced disease stage ($p<0.05$). Also size of tumour and HER2/neu over expression showed positive correlation ($p<0.05$). There is also positive correlation between tumour grade and HER2/neu expression ($p<0.001$). They observed that right colonic tumours constitutes 45.6% whereas left colorectal tumours constitutes 54.4%. In his study, mostly moderately differentiated and poorly differentiated carcinomas overexpressed HER2/neu.

Li et al [42] evaluated HER2/neu expression in 317 colorectal carcinoma specimens. Among them, 49 cases (15.5%) showed HER2/neu positivity with strong positivity in 7 cases. There was association between HER2/neu expression and tumour size ($p<0.05$). But there was no association with clinicopathological features.

According to a study by Lazaris et al [43], HER2/neu expression was found in 36% of cases and they reported it as a predictor of poor outcome.

OBSERVATION

&

RESULTS

OBSERVATION AND RESULTS

Histopathological examination of 50 cases of colorectal carcinoma were studied and staging was done based on TNM classification. The following are the results.

Table -1

Age presentation of colorectal carcinoma

Age	Male	Female	Total
21 to 30	0	0	0
31 to 40	3	3	6
41 to 50	5	8	13
51 to 60	8	7	15
61 to 70	5	5	10
71 to 80	6	0	6
Total	27	23	50

From table no.1, it was evident that age of colorectal carcinoma patients range from 35 to 75 years. Males are more affected than females.

CHART - 1

AGE PRESENTATION OF COLORECTAL CARCINOMA

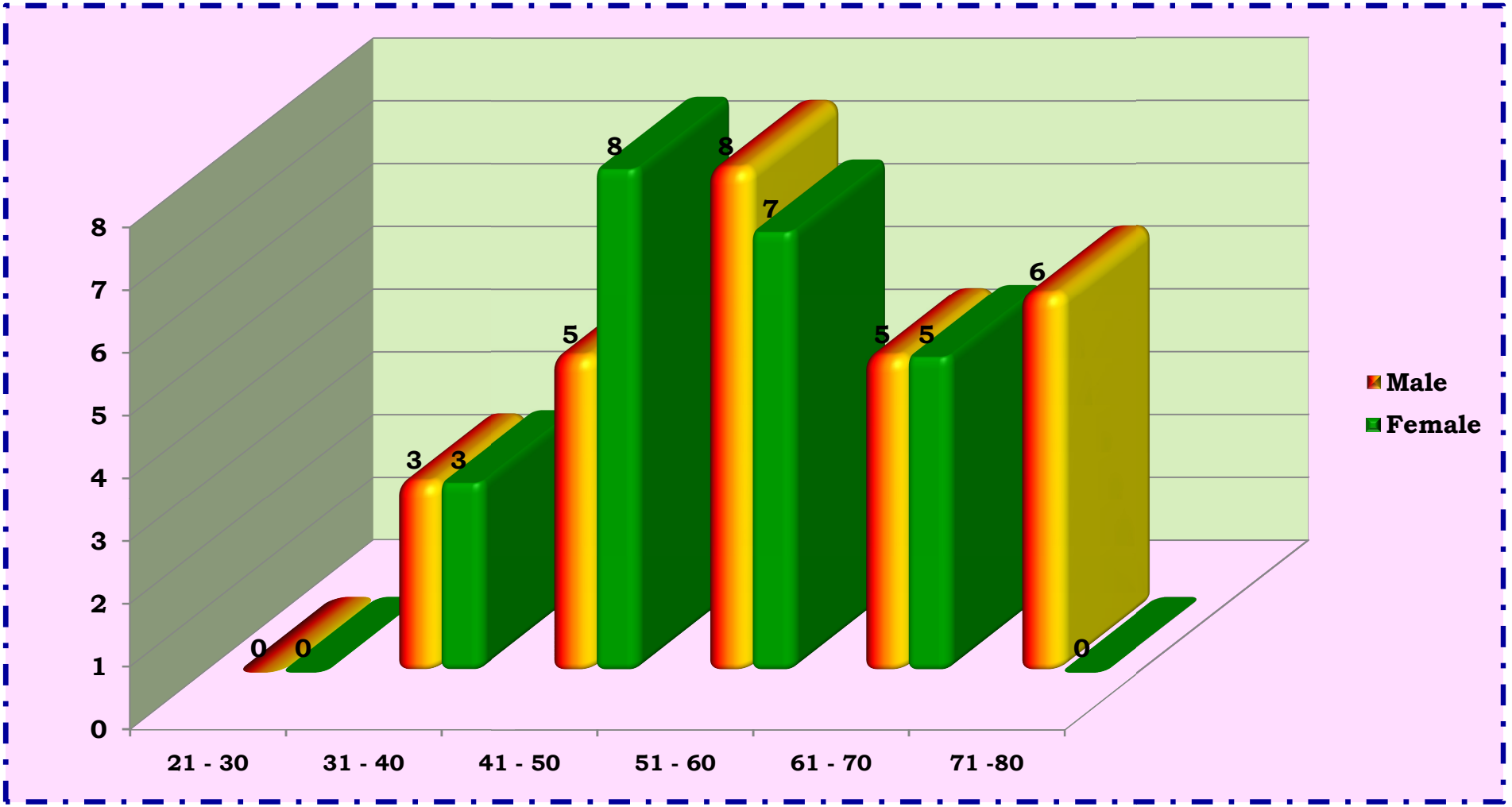


Table -2

Clinical presentation of colorectal carcinoma

Symptoms	No of Cases	Percentage
Altered Bowel Habits	35	70%
Abdominal Pain	20	40%
Bleeding per rectum	15	30%
Weight loss	8	16%
Intestinal Obstruction	8	16%

Table No.2 shows that, the most common clinical presentation of colorectal carcinoma patients in our study was altered bowel habits (70%) and abdominal pain (40%).

CHART -2

CLINICAL PRESENTATION OF COLORECTAL CARCINOMA

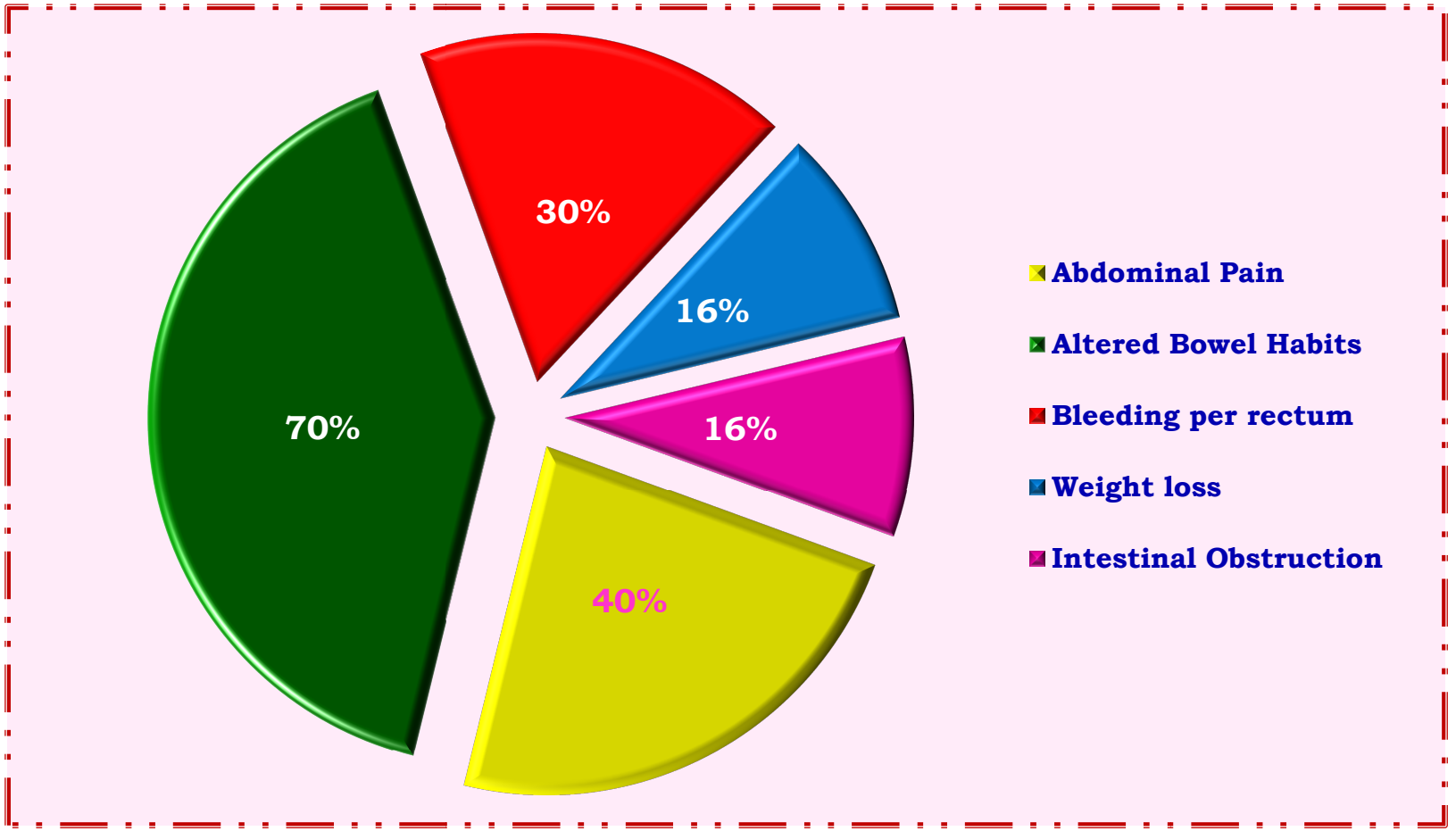


Table - 3

Colonoscopic findings of tumours of colorectal region

Colonoscopic findings	No. of cases	Percentage
Ulceroproliferative	37	74%
Narrowing of Lumen	10	20%
Polyp	3	6%
Total	50	100%

Above table.3 shows that grossly, ulceroproliferative growth was the common macroscopic finding found in colorectal carcinomas.

CHART - 3

COLONOSCOPIC FINDINGS OF TUMOURS OF COLORECTAL REGION

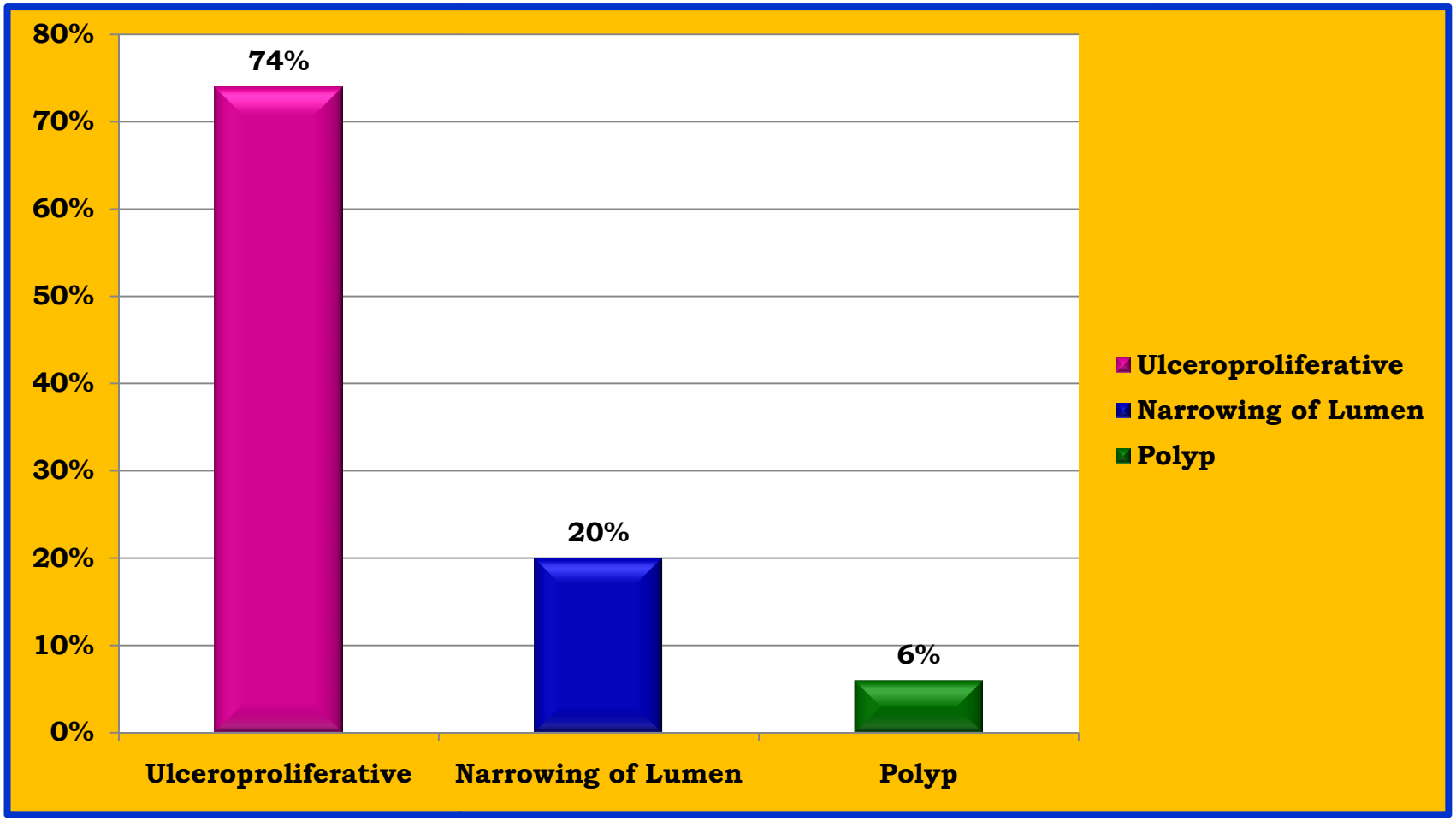


Table - 4

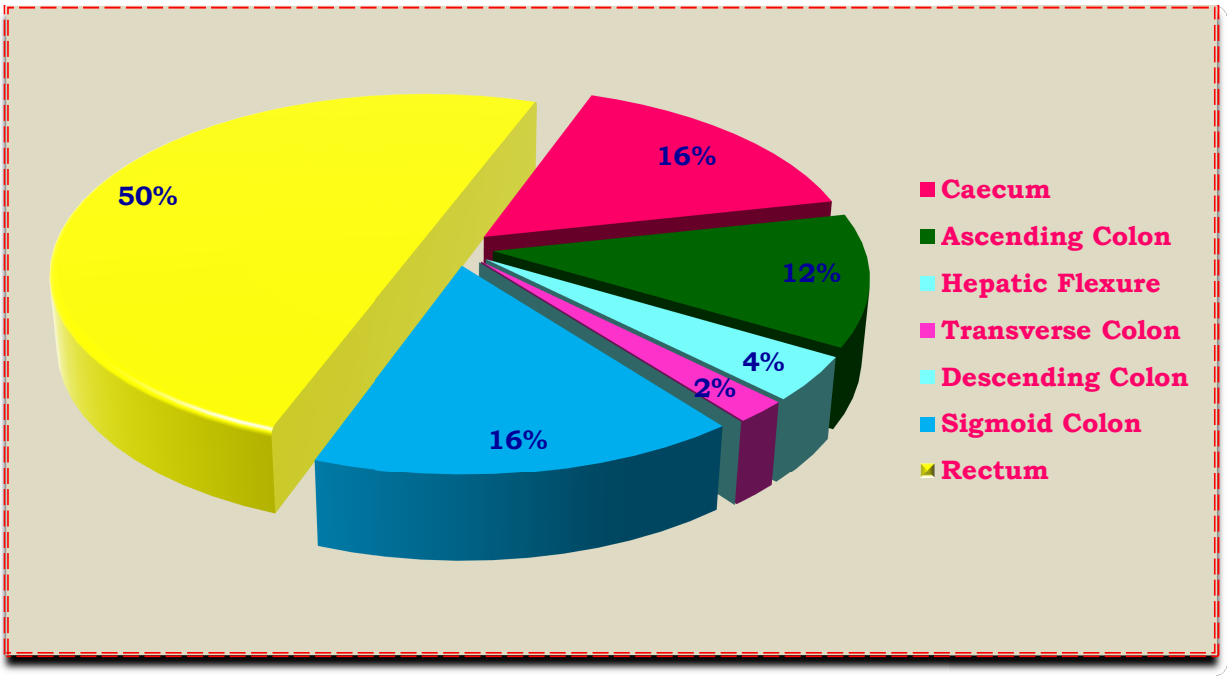
Site distribution of colorectal carcinomas

Site	No.of cases			Percentage
	Male	Female	Total	
Caecum	5	3	8	16%
Ascending Colon	4	2	6	12%
Hepatic Flexure	1	1	2	4%
Transverse Colon	1	0	1	2%
Descending Colon	0	0	0	0%
Sigmoid Colon	4	4	8	16%
Rectum	12	13	25	50%
Total	27	23	50	100%

From table no.4, it was evident that the most common site affected by colorectal carcinomas was rectum (50%), followed by caecum (16%) and sigmoid colon (16%). In both sexes, rectum was commonly affected in colorectal carcinoma.

CHART - 4

SITE DISTRIBUTION OF COLORECTAL CARCINOMAS



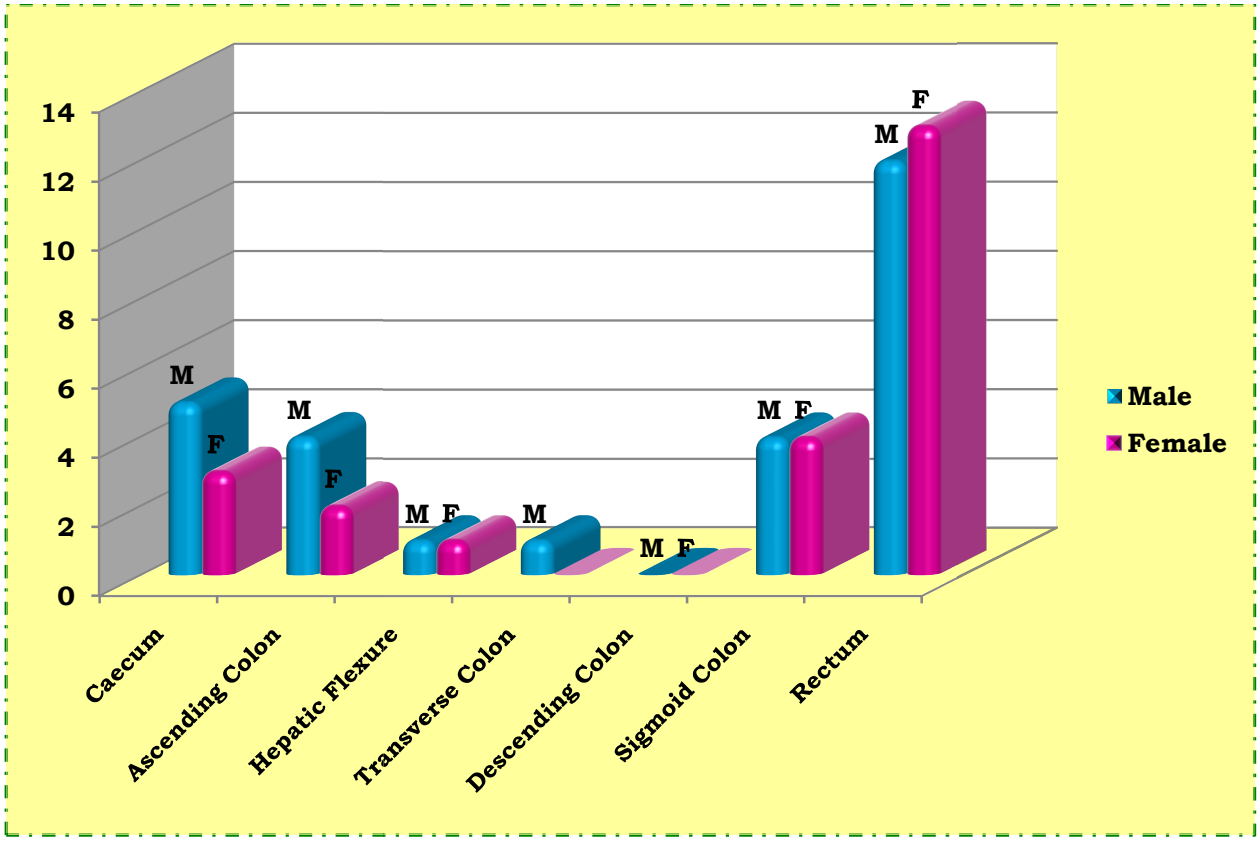


Table - 5

Correlation of site distribution of tumours of colorectal region with age

Site	21-30	31-40	41-50	51-60	61-70	71-80	Total
Caecum	0	3	1	3	1	0	8
Ascending Colon	0	0	1	4	1	0	6
Hepatic Flexure	0	0	0	0	1	1	2
Transverse Colon	0	0	0	0	1	0	1
Descending Colon	0	0	0	0	0	0	0
Sigmoid Colon	0	0	2	4	1	1	8
Rectum	0	4	8	5	5	3	25
Total	0	7	12	16	10	5	50

From above table no.5, it was evident that the most common site affected in all age groups was rectum.

CHART - 5

CORRELATION OF SITE DISTRIBUTION OF TUMOURS OF COLORECTAL REGION WITH AGE

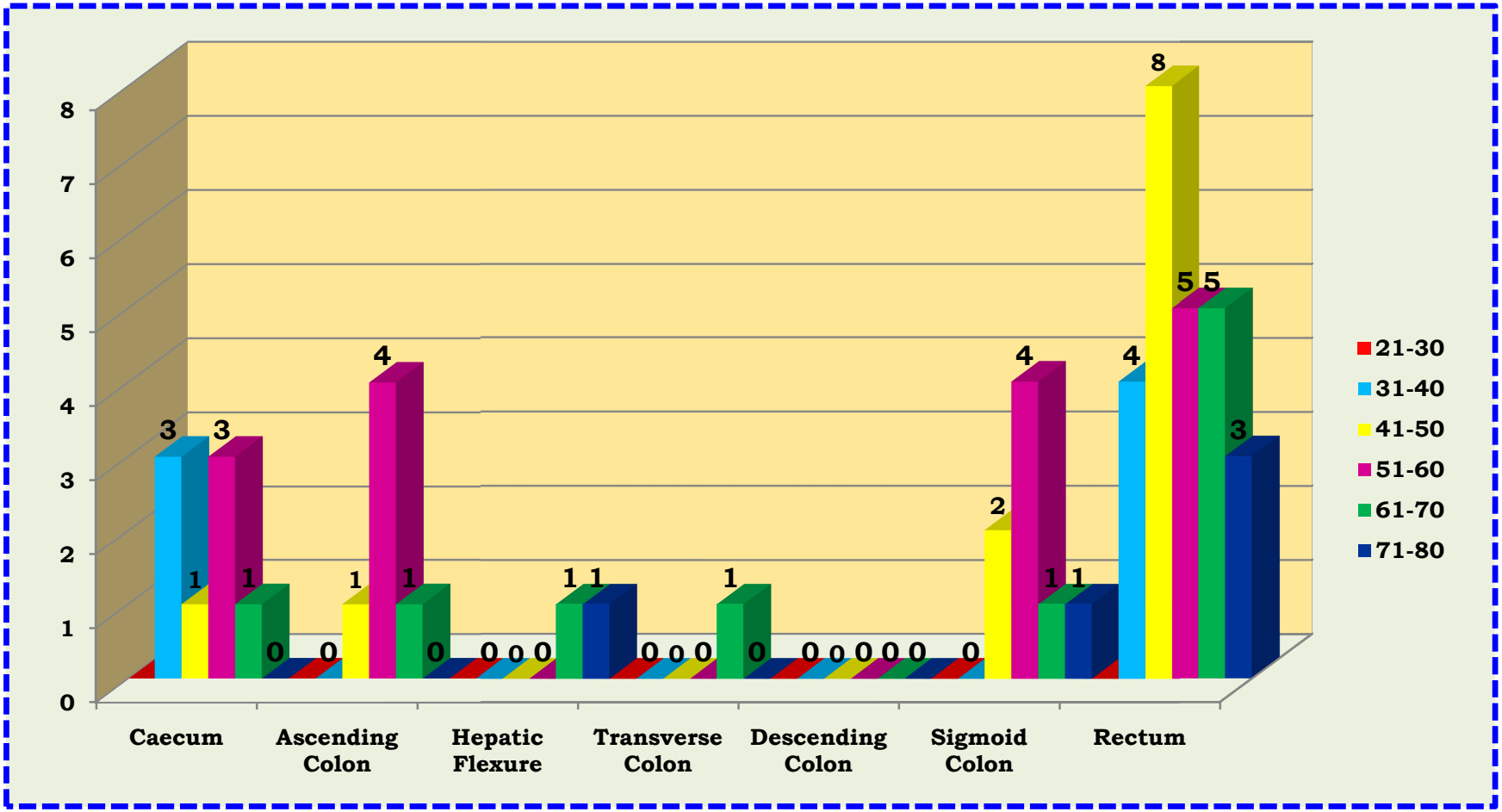


Table - 6

Size distribution of tumours of colorectal region

Size	No. of Cases
Upto 5Cm	32
6 - 10 Cm	14
>10 Cm	4
Total	50

In this study, most of the tumours of colorectal region are less than 5 cm in size.

CHART - 6

SIZE DISTRIBUTION OF TUMOURS OF COLORECTAL REGION

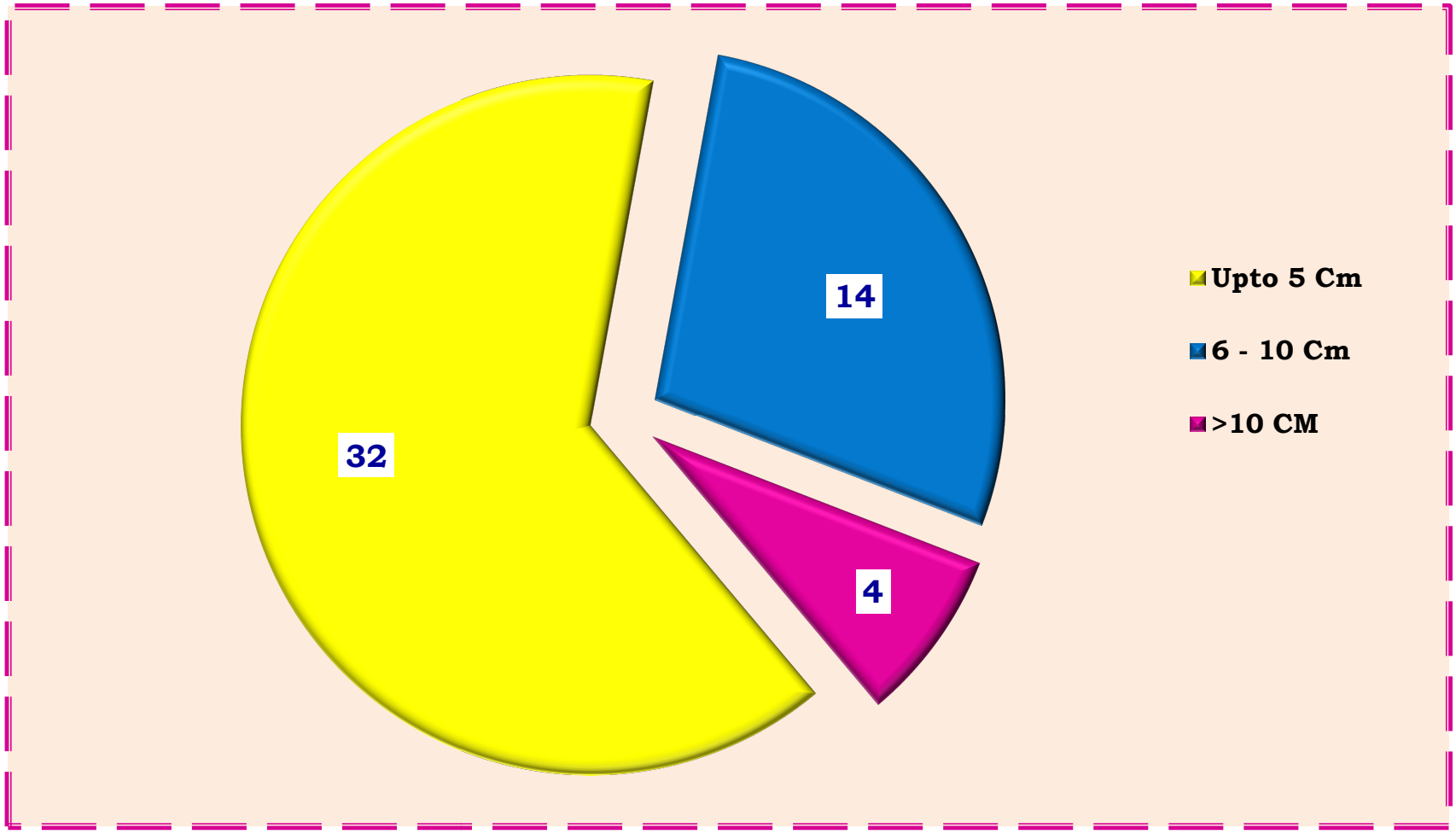


Table - 7

Correlation of size distribution of tumours of colorectal region with site

Site	Size			Total
	upto 5 cm	6-10 cm	>10cm	
Caecum	5	2	1	8
Ascending Colon	4	2	0	6
Hepatic Flexure	1	0	1	2
Transverse Colon	0	0	1	1
Descending Colon	0	0	0	0
Sigmoid Colon	6	2	0	8
Rectum	16	8	1	25
Total	32	14	4	50

From table no.6&7, it was inferred that tumours less than 5cm size are more common in all the regions of large intestine. This was mainly due to increased availability of medical services and earlier diagnosis of colorectal carcinoma.

CHART - 7

Correlation of size distribution of tumours of colorectal region with site

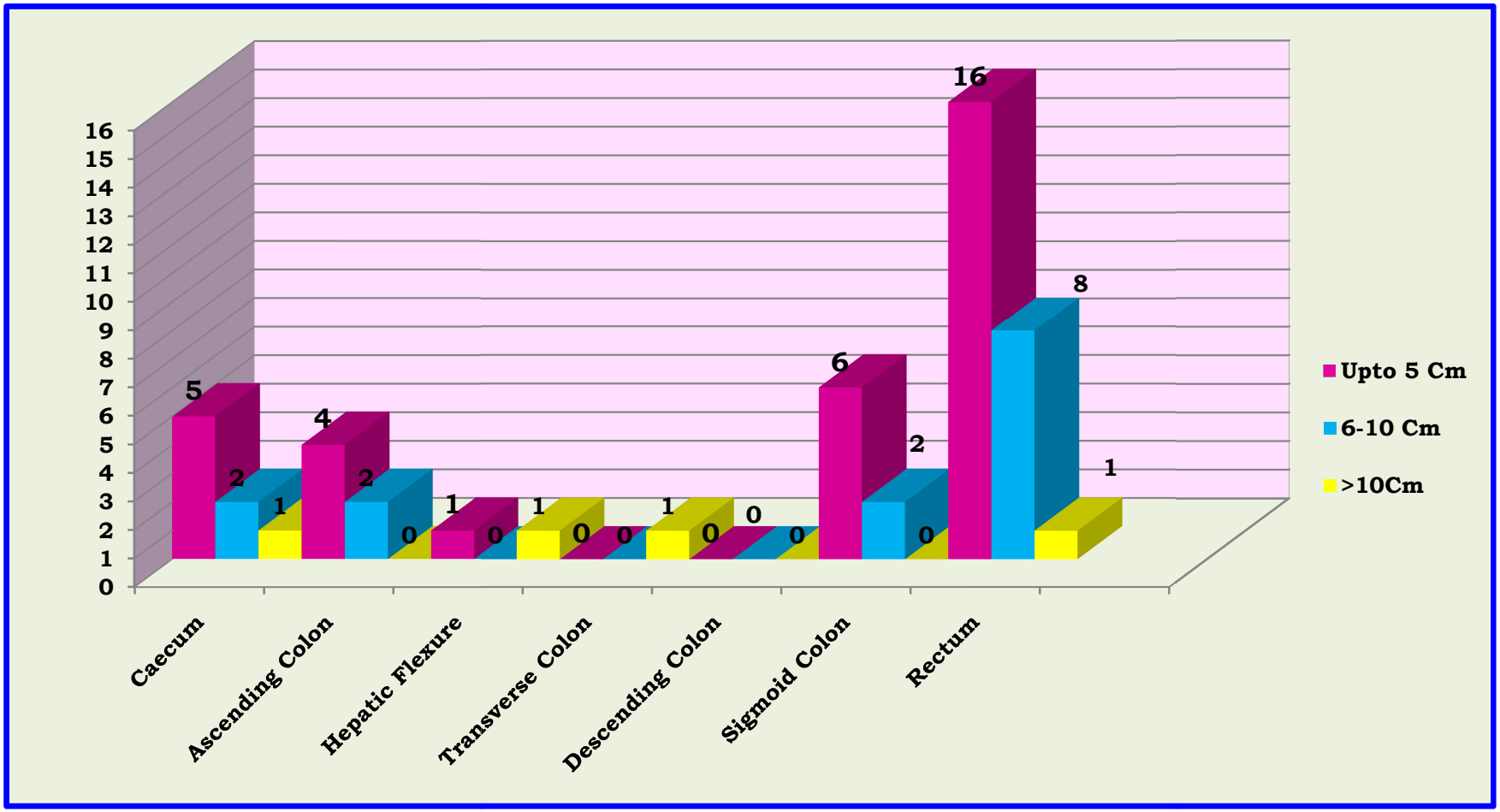


Table - 8

Incidence of conventional and mucinous adenocarcinomas

Adenocarcinomas	No of cases	Percentage(%)
Conventional adenocarcinoma	44	88
Mucinous carcinoma	6	12

CHART - 8

Incidence of Mucinous and Non-Mucinous adenocarcinomas

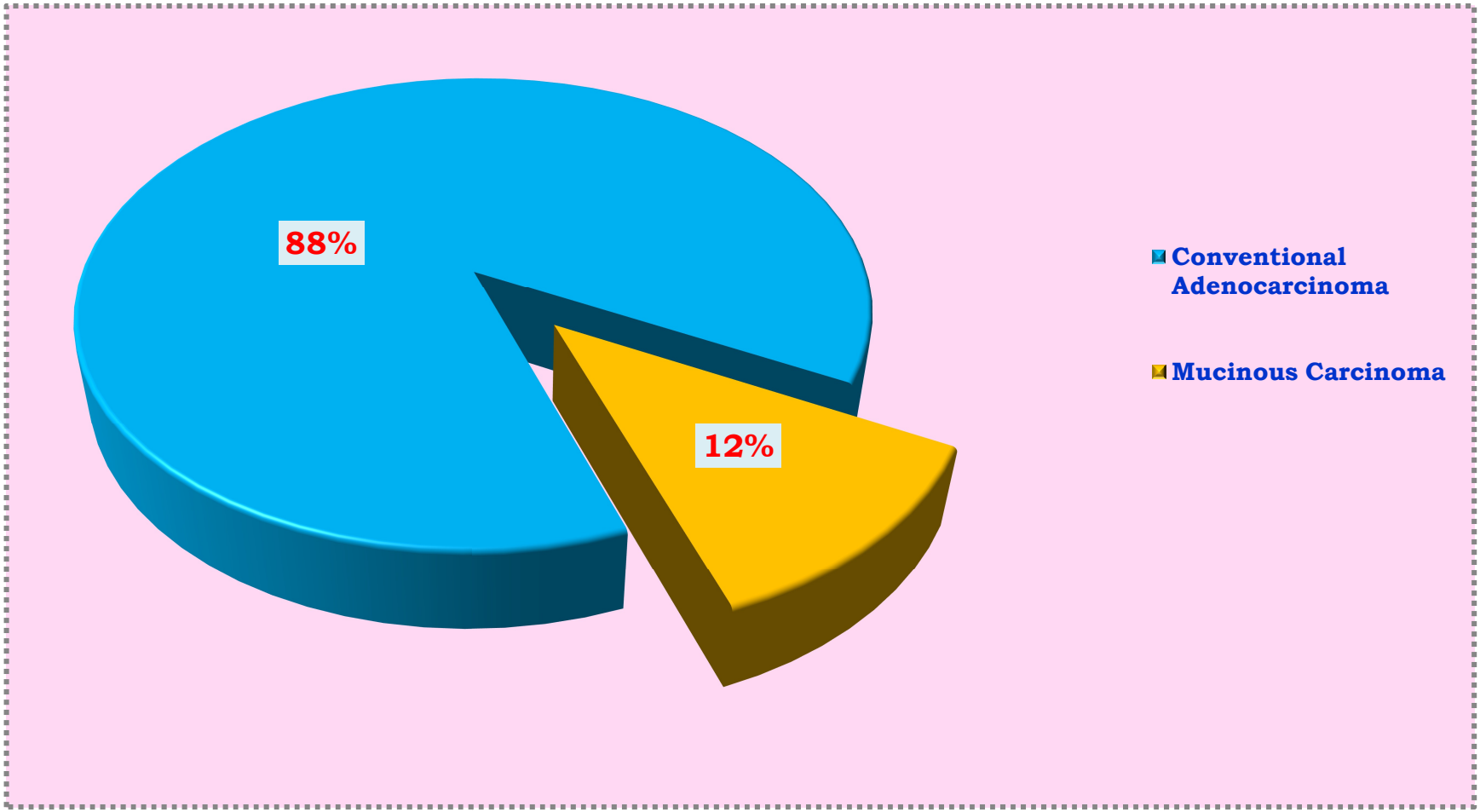


Table 9

Histological grades of colorectal adenocarcinomas

Grade	No. of Cases
Grade I	10
Grade II	33
Grade III	1

From above table no.9, it was inferred that Grade II tumours (moderately differentiated carcinomas) are more frequent than other grades of colorectal carcinomas in this study.

CHART - 9

Histological grades of colorectal adenocarcinomas

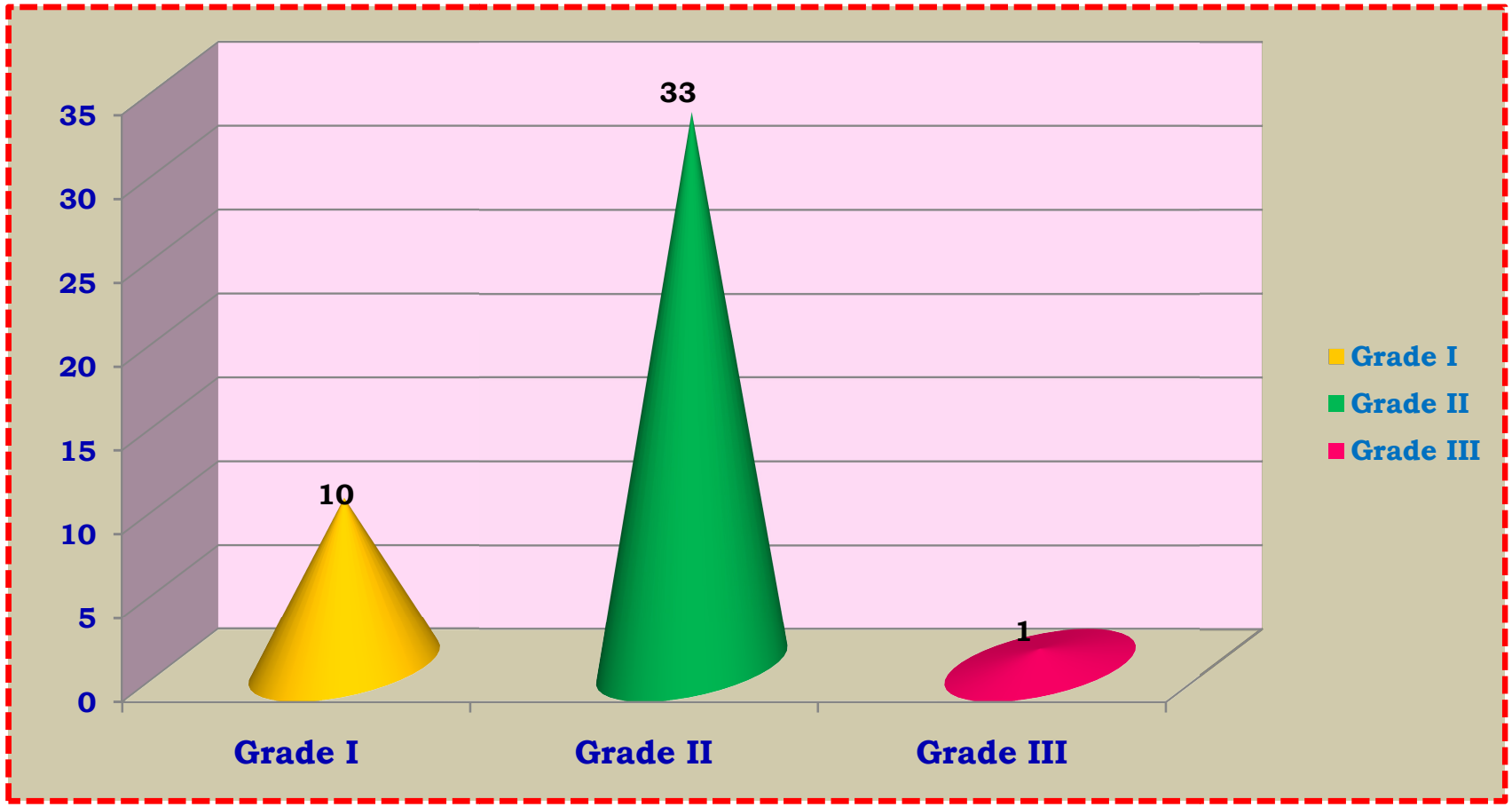


Table - 10

Correlation of age and sex distribution of colorectal carcinoma patients with histological grade

Age	Grade I		
	Male	Female	Total
21 to 30	0	0	0
31 to 40	0	2	2
41 to 50	2	0	2
51 to 60	3	1	4
61 to 70	1	1	2
71 to 80	0	0	0
Total	6	4	10

CHART - 10

Correlation of age and sex distribution of colorectal carcinoma patients with histological grade

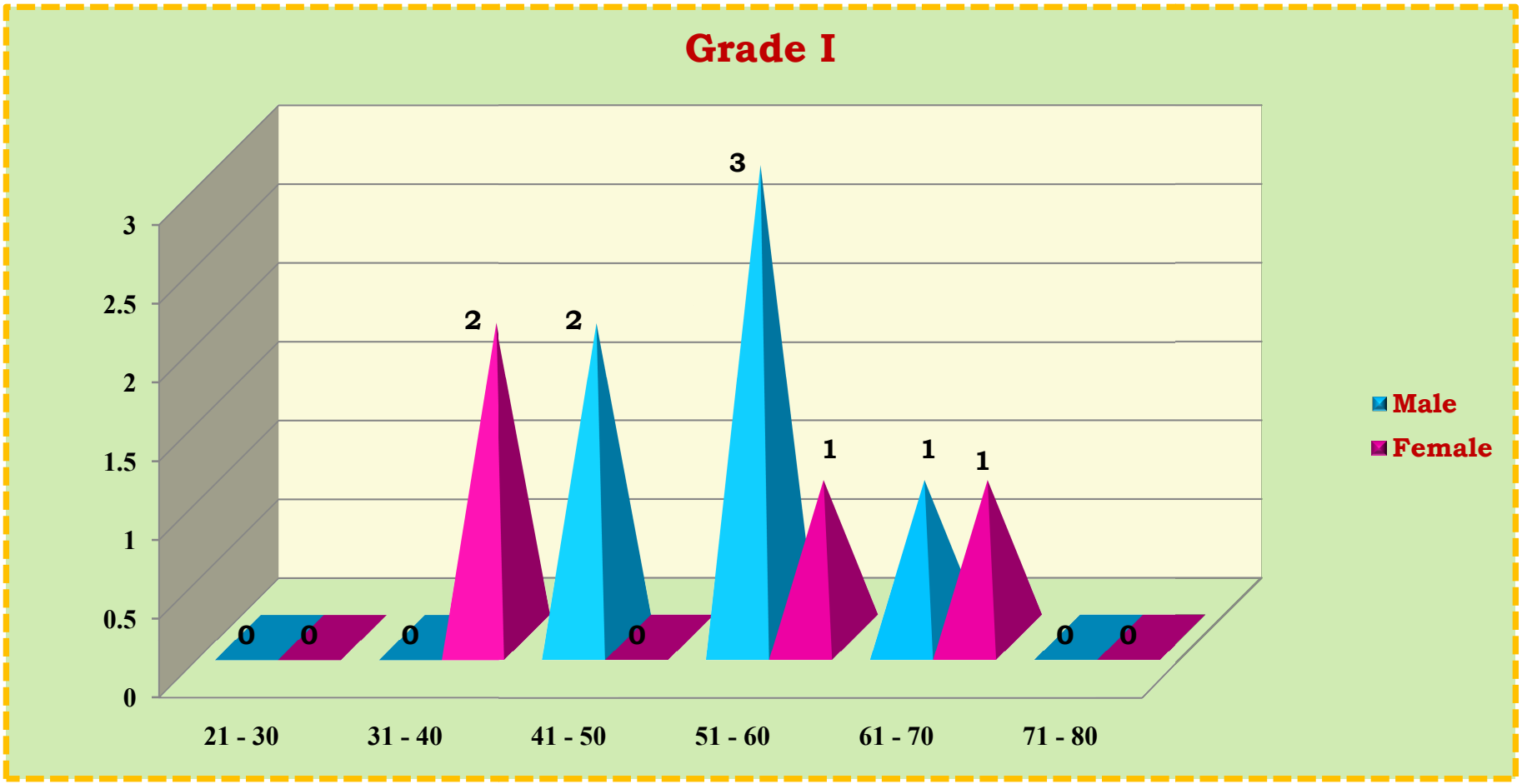


Table - 11

Correlation of age and sex distribution of colorectal carcinoma patients with histological grade

Age	Grade II		
	Male	Female	Total
21 to 30	0	0	0
31 to 40	3	0	3
41 to 50	3	7	10
51 to 60	5	5	10
61 to 70	2	2	4
71 to 80	6	0	6
Total	19	14	33

Table - 12

Correlation of age and sex distribution of colorectal carcinoma patients with histological grade

Age	Grade III		
	Male	Female	Total
21 to 30	0	0	0
31 to 40	0	0	0
41 to 50	0	0	0
51 to 60	0	1	1
61 to 70	0	0	0
71 to 80	0	0	0
Total	0	1	1

CHART - 11

Correlation of age and sex distribution of colorectal carcinoma patients with histological grade

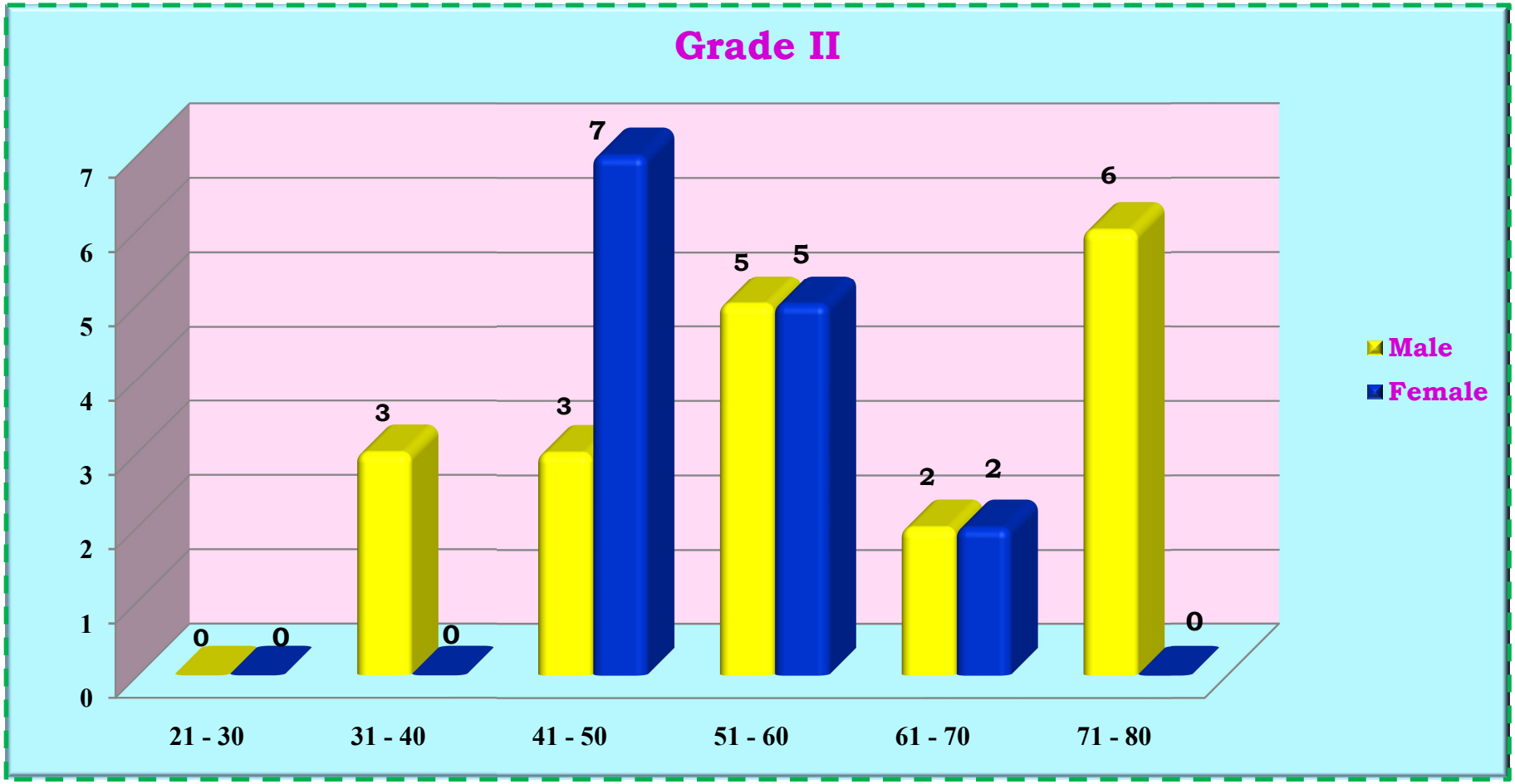


CHART - 12

Correlation of age and sex distribution of colorectal carcinoma patients with histological grade

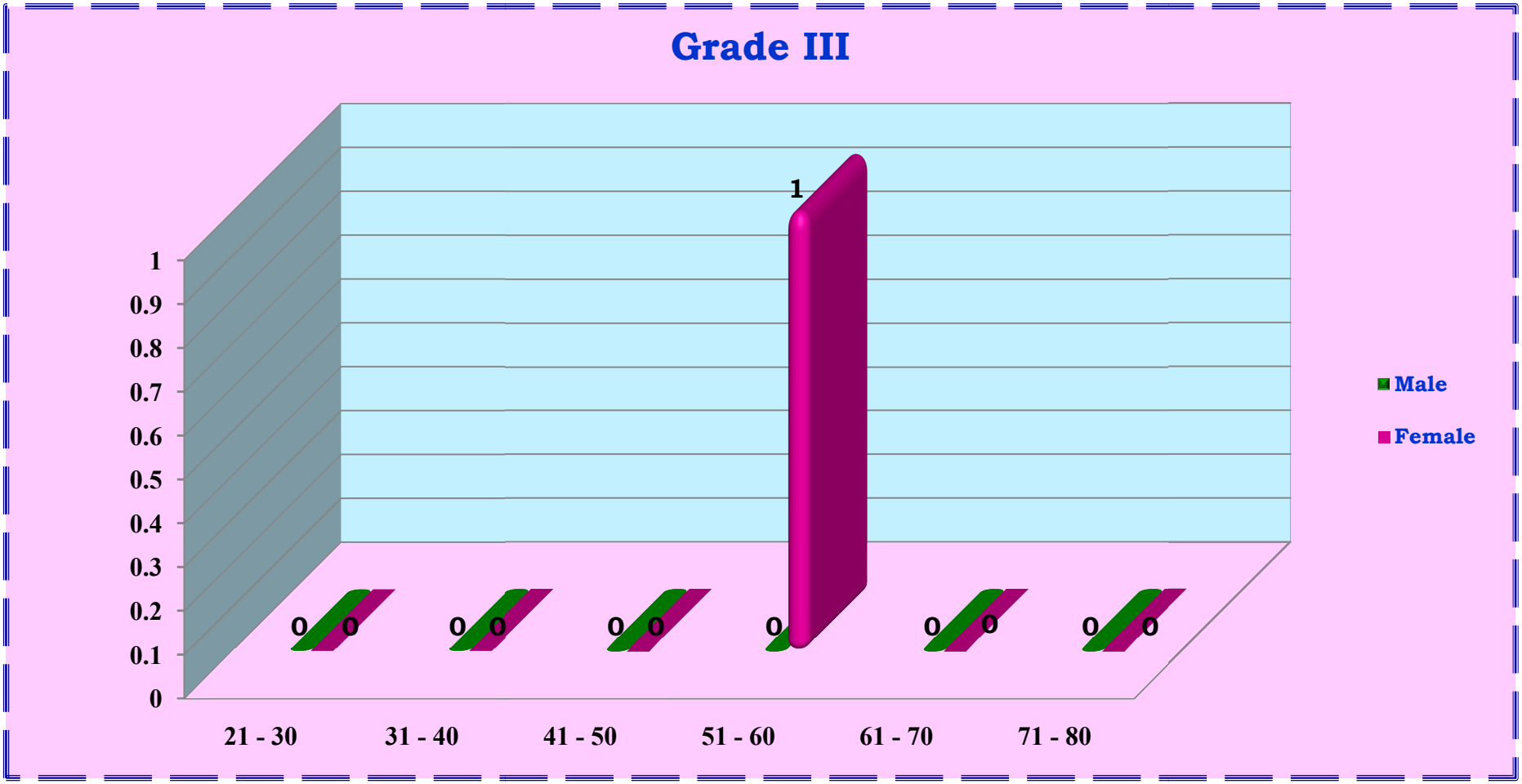


Table - 13

Correlation of age and sex distribution of colorectal carcinoma patients with mucinous carcinoma

Age	Mucinous Carcinoma		
	Male	Female	Total
21 to 30	0	0	0
31 to 40	0	2	2
41 to 50	0	0	0
51 to 60	0	0	0
61 to 70	2	2	4
71 to 80	0	0	0
Total	2	4	6

From table no.10-13, it was inferred that low grade tumours i.e. grade I & grade II tumours occur more commonly in males. High grade tumours i.e. grade III and mucinous carcinomas are commonly seen in females.

CHART - 13

Correlation of age and sex distribution of colorectal carcinoma patients with Mucinous Carcinoma

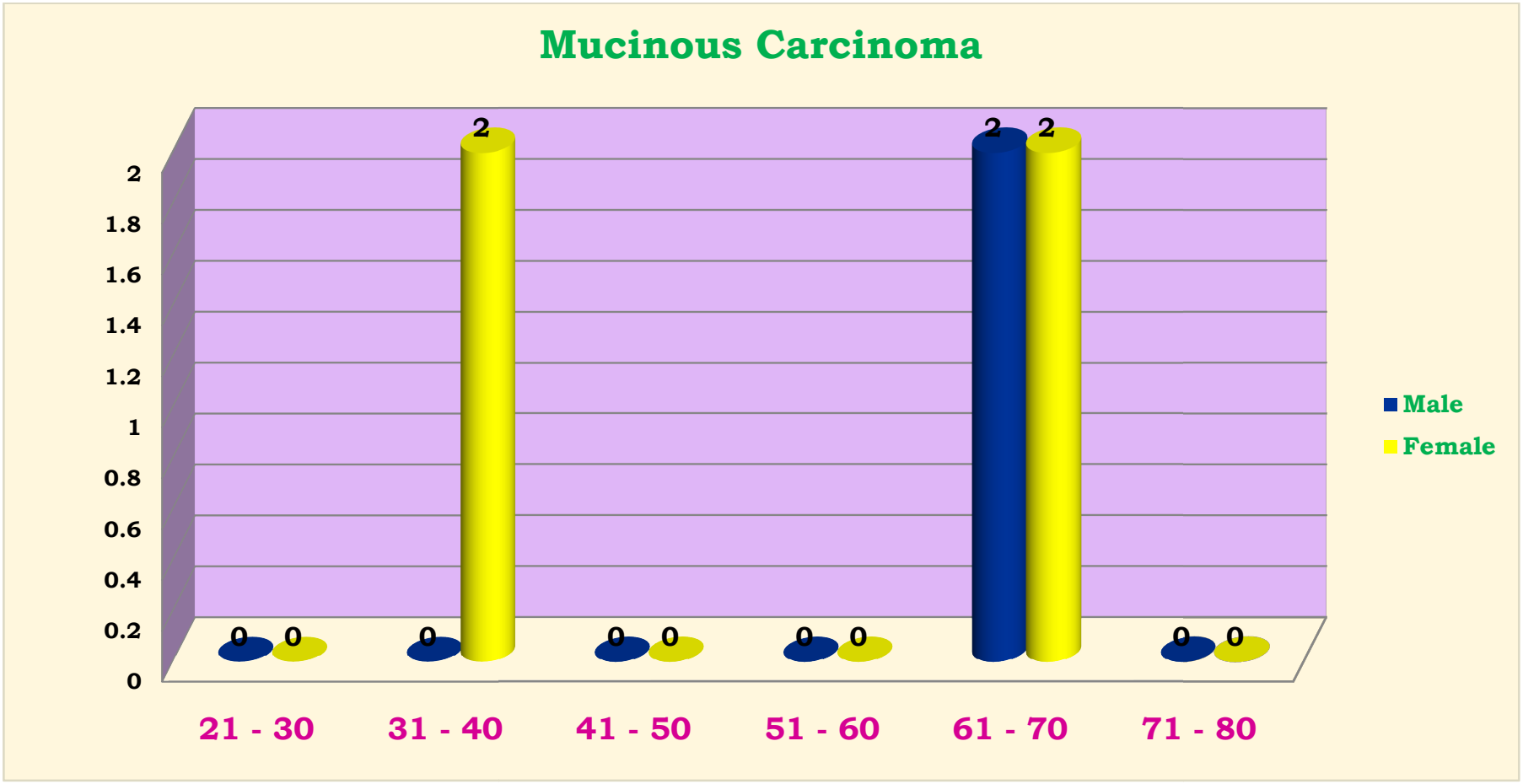


Table-14

Stage distribution of colorectal carcinomas

Stage	No of Cases
Stage I	17
Stage II	12
Stage III	18
Stage IV	3

Table no.14 shows that stage III tumours are more when compared to other stages in this study.

CHART - 14

Stage distribution of colorectal carcinomas

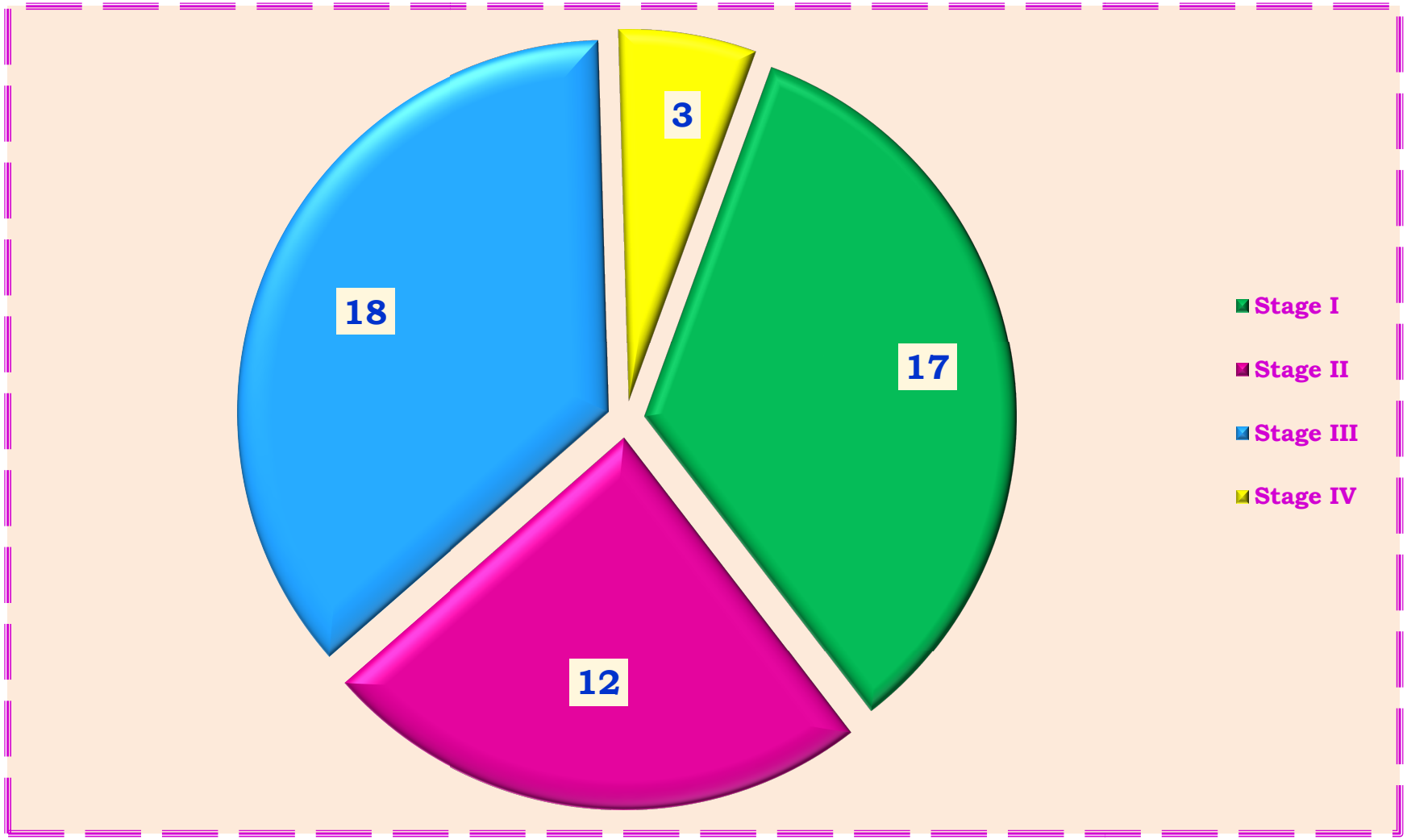


Table - 15

Correlation of age and sex distribution of colorectal carcinoma patients with stage

Age	Stage I		
	Male	Female	Total
21 to 30	0	0	0
31 to 40	3	1	4
41 to 50	0	2	2
51 to 60	4	2	6
61 to 70	1	3	4
71 to 80	1	0	1
Total	9	8	17

CHART - 15

Correlation of age and sex distribution of colorectal carcinoma patients with stage

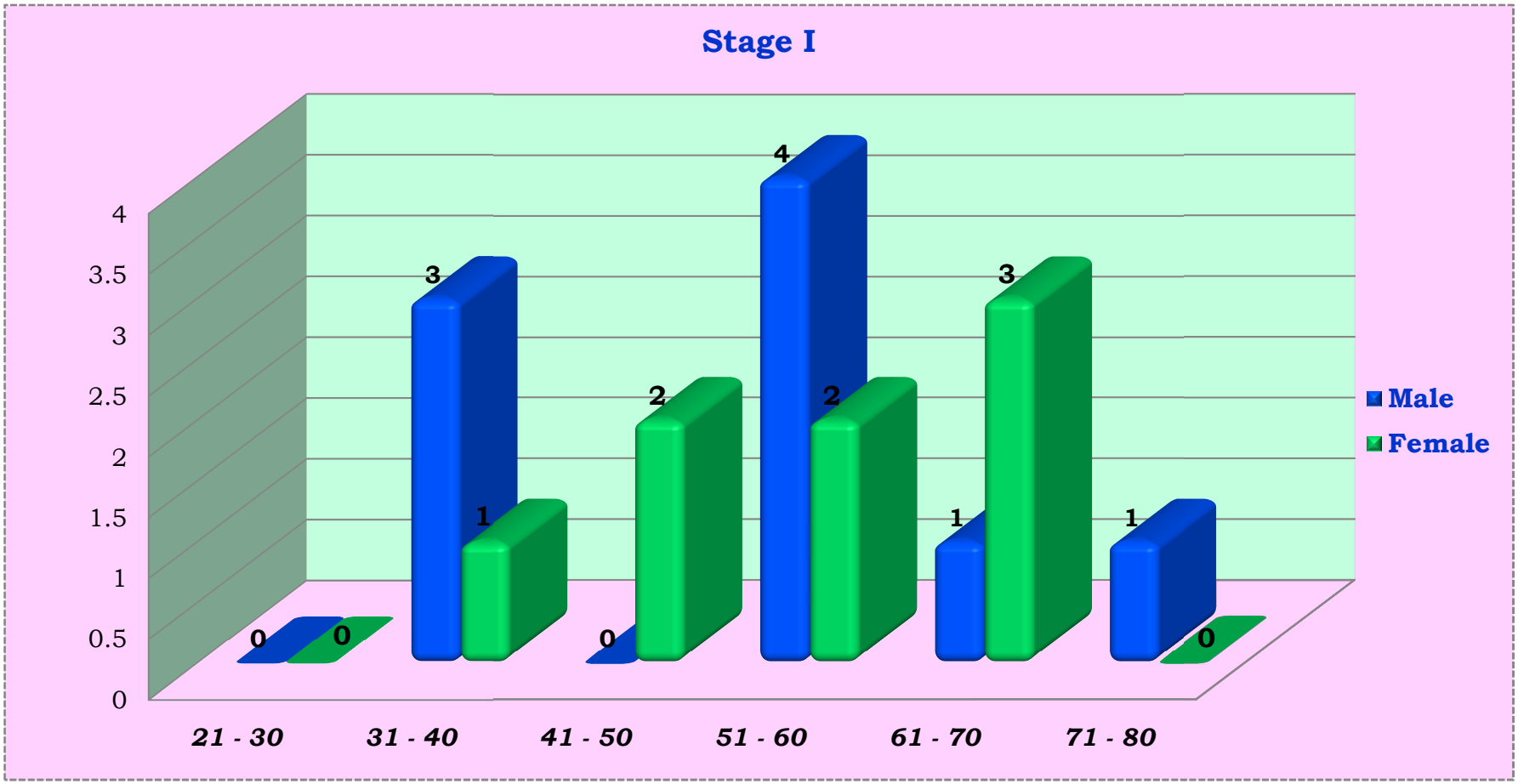


Table - 16

Correlation of age and sex distribution of colorectal carcinoma patients with stage

Age	Stage II		
	Male	Female	Total
21 to 30	0	0	0
31 to 40	0	1	1
41 to 50	0	2	2
51 to 60	2	2	4
61 to 70	1	1	2
71 to 80	3	0	3
Total	6	6	12

Table - 17

Correlation of age and sex distribution of colorectal carcinoma patients with stage

Age	Stage III		
	Male	Female	Total
21 to 30	0	0	0
31 to 40	0	1	1
41 to 50	5	2	7
51 to 60	2	2	4
61 to 70	3	1	4
71 to 80	2	0	2
Total	12	6	18

CHART - 16

Correlation of age and sex distribution of colorectal carcinoma patients with stage

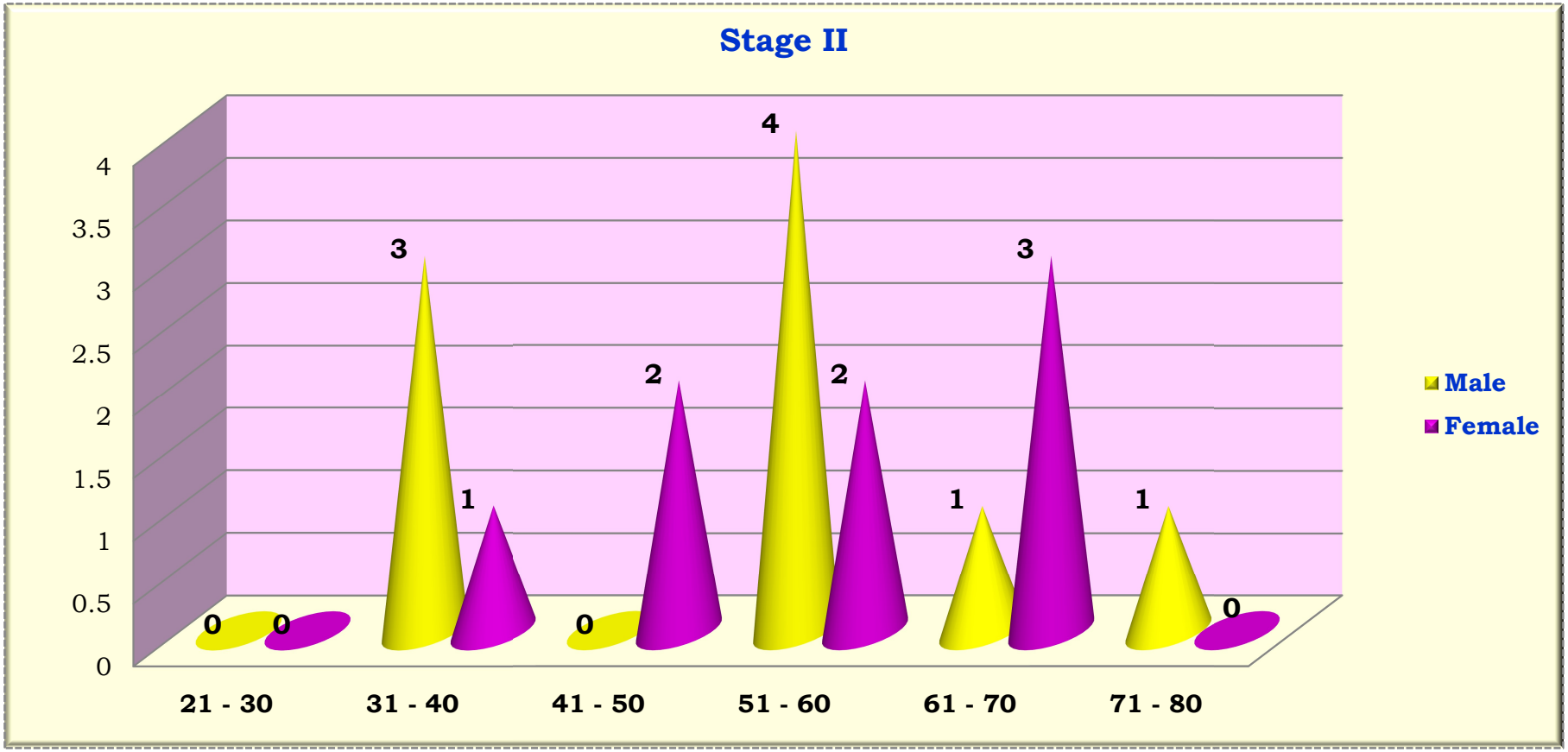


CHART - 17

Correlation of age and sex distribution of colorectal carcinoma patients with stage

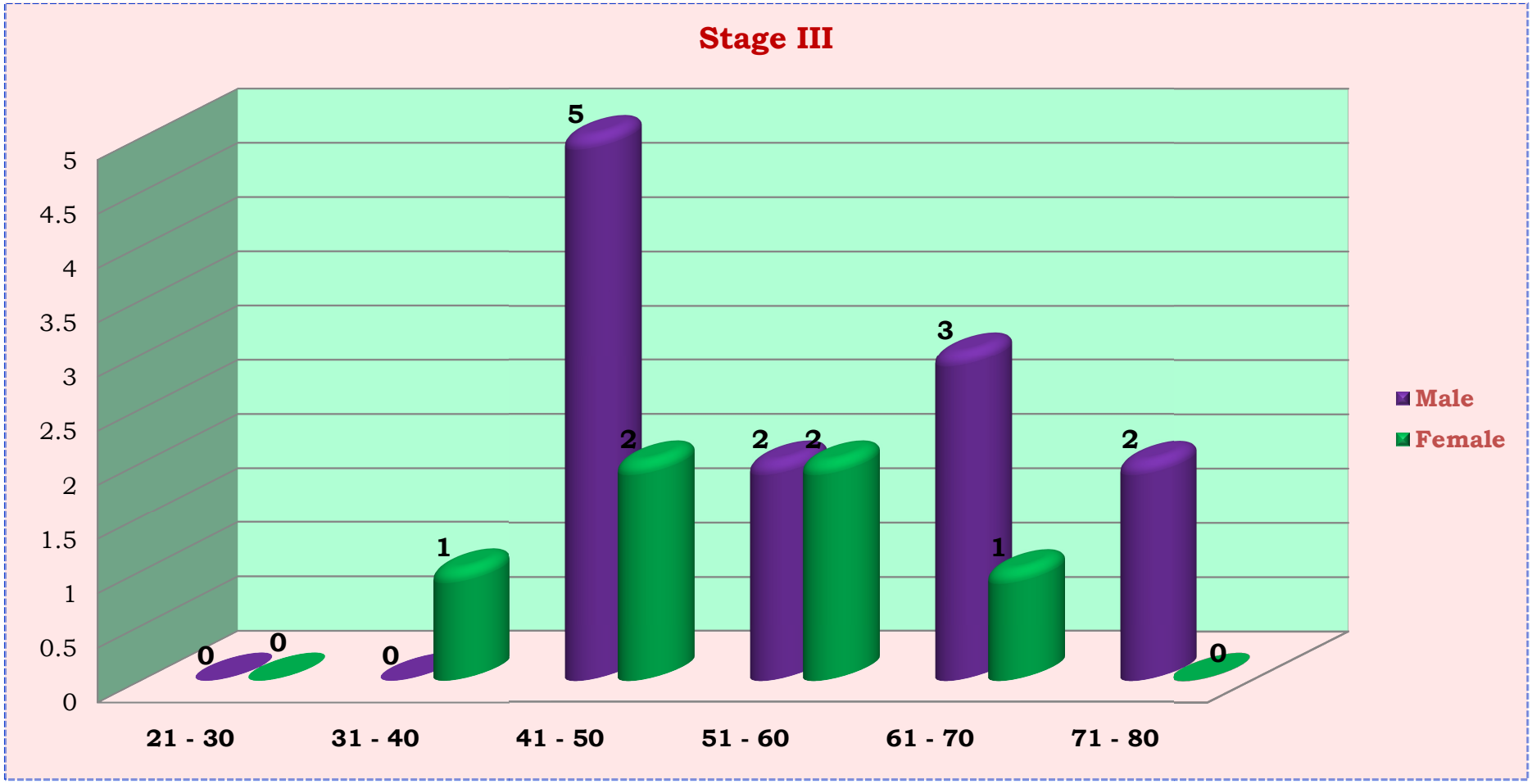


Table - 18

Correlation of age and sex distribution of colorectal carcinoma patients with stage

Age	Stage IV		
	Male	Female	Total
21 to 30	0	0	0
31 to 40	0	0	0
41 to 50	0	2	2
51 to 60	0	1	1
61 to 70	0	0	0
71 to 80	0	0	0
Total	0	3	3

CHART - 18

Correlation of age and sex distribution of colorectal carcinoma patients with stage

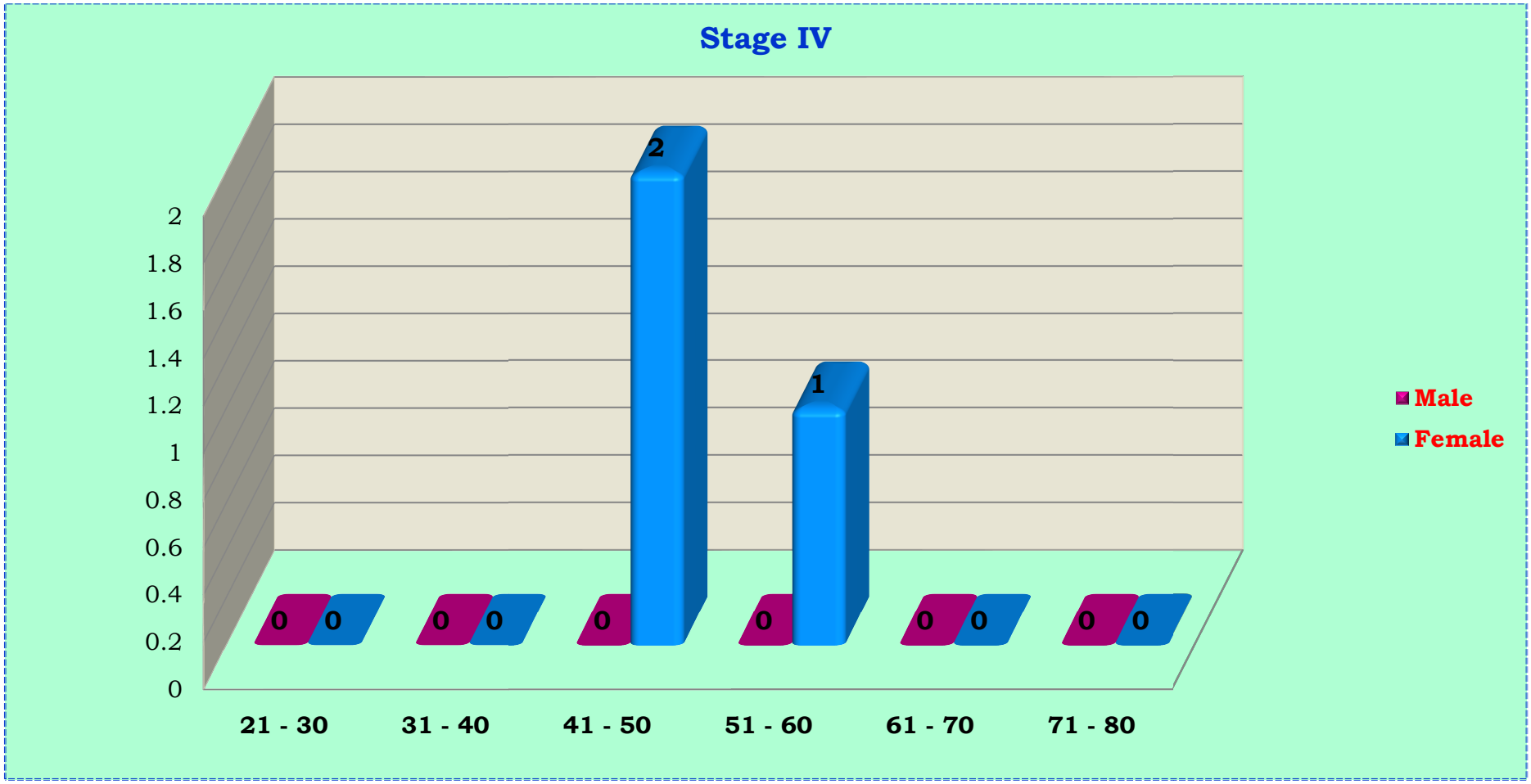


Table - 19

HER2/neu expression in colorectal carcinomas

S.No	Biopsy No.	Histological Grade	Stage	HER2/neu score
1	14/16	I	III	2+
2	451/16	III	II	0
3	1153/16	II	III	0
4	1567/16	II	I	0
5	1571/16	I	II	0
6	1799/16	II	IV	0
7	2368/16	II	II	0
8	2624/16	II	III	1+
9	2671/16	I	III	0
10	3154/16	II	II	2+
11	3947/16	II	III	2+
12	4131/16	II	II	0
13	4147/16	I	I	1+
14	4719/16	II	I	0
15	115/17	MUCINOUS CARCINOMA	III	3+
16	289/17	II	III	0
17	530/17	II	III	2+
18	587/17	MUCINOUS CARCINOMA	III	0
19	694/17	II	IV	0
20	706/17	II	I	2+

S.No	Biopsy No.	Histological Grade	Stage	HER2/neu score
21	1059/17	II	II	0
22	1185/17	I	II	0
23	1342/17	MUCINOUS CARCINOMA	III	1+
24	1591/17	II	III	0
25	1684/17	II	III	0

Table - 20

HER2/neu expression in colorectal adenocarcinomas

HER2/neu Expression	Positive	Negative	Total
Conventional adenocarcinomas	5	17	22
Mucinous carcinomas	1	2	3
Total	6	19	25

CHART - 19

HER2/neu expression in colorectal adenocarcinomas

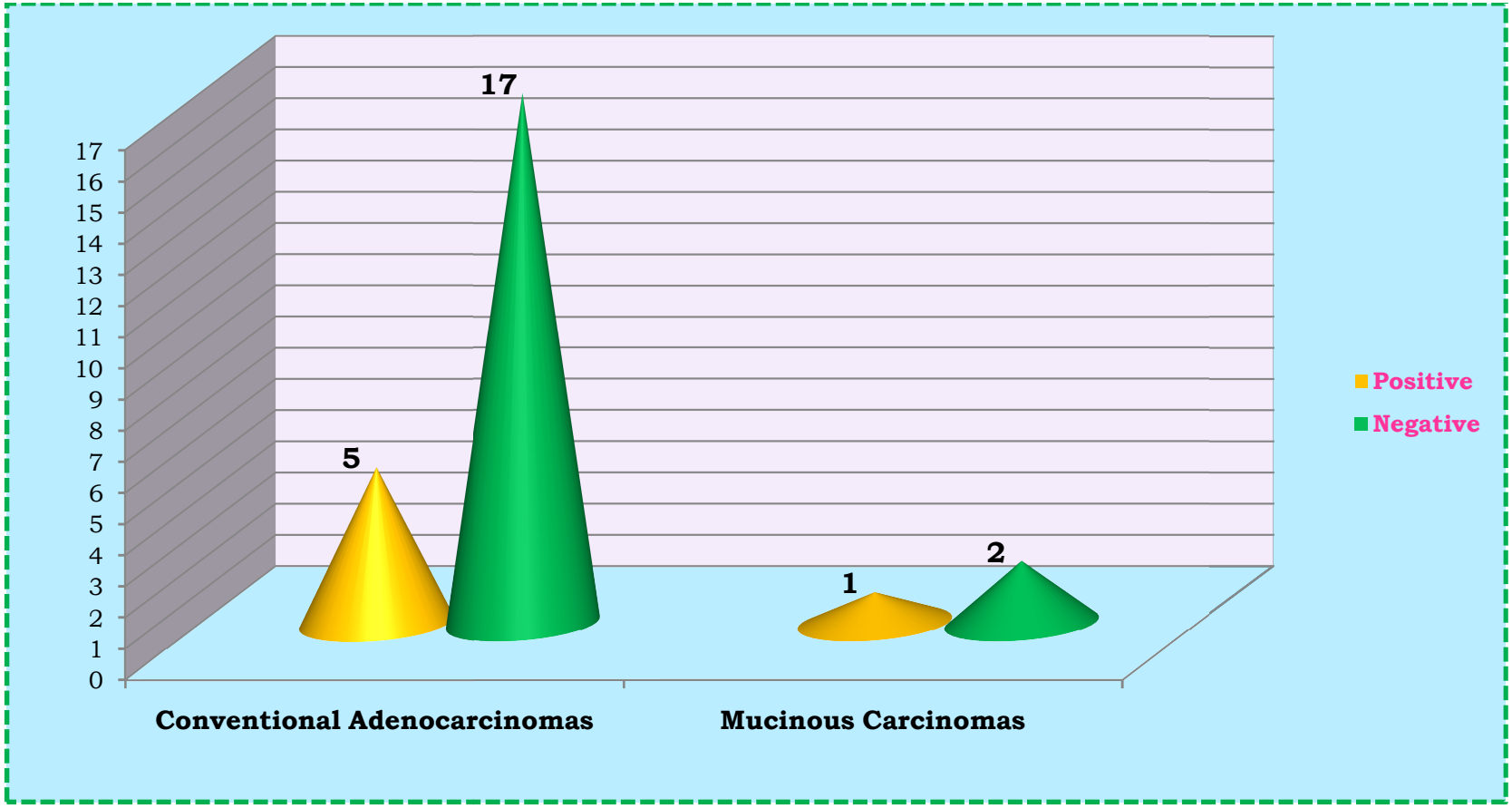


Table - 21

HER2/neu scoring in colorectal carcinomas

Adenocarcinomas	**HER2/neu score**				**Total**
	0	**1**	**2**	**3**	
Conventional adenocarcinomas	15	2	5	0	22
Mucinous adenocarcinomas	1	1	0	1	3
Total	16	3	5	1	25

In this study, HER2/neu expression of moderate membranous positivity (2+) and strong membranous positivity (3+) was considered as positive. From above table no.20 and 21, it was inferred that, HER2/neu overexpression was seen in 6 cases. Among them, five cases showing moderate membranous positivity (2+) were conventional adenocarcinomas and one case showing strong membranous positivity (3+) was mucinous carcinoma. However statistical analysis did not prove this association(p=0.114).

CHART - 20

HER2/neu scoring in colorectal carcinomas

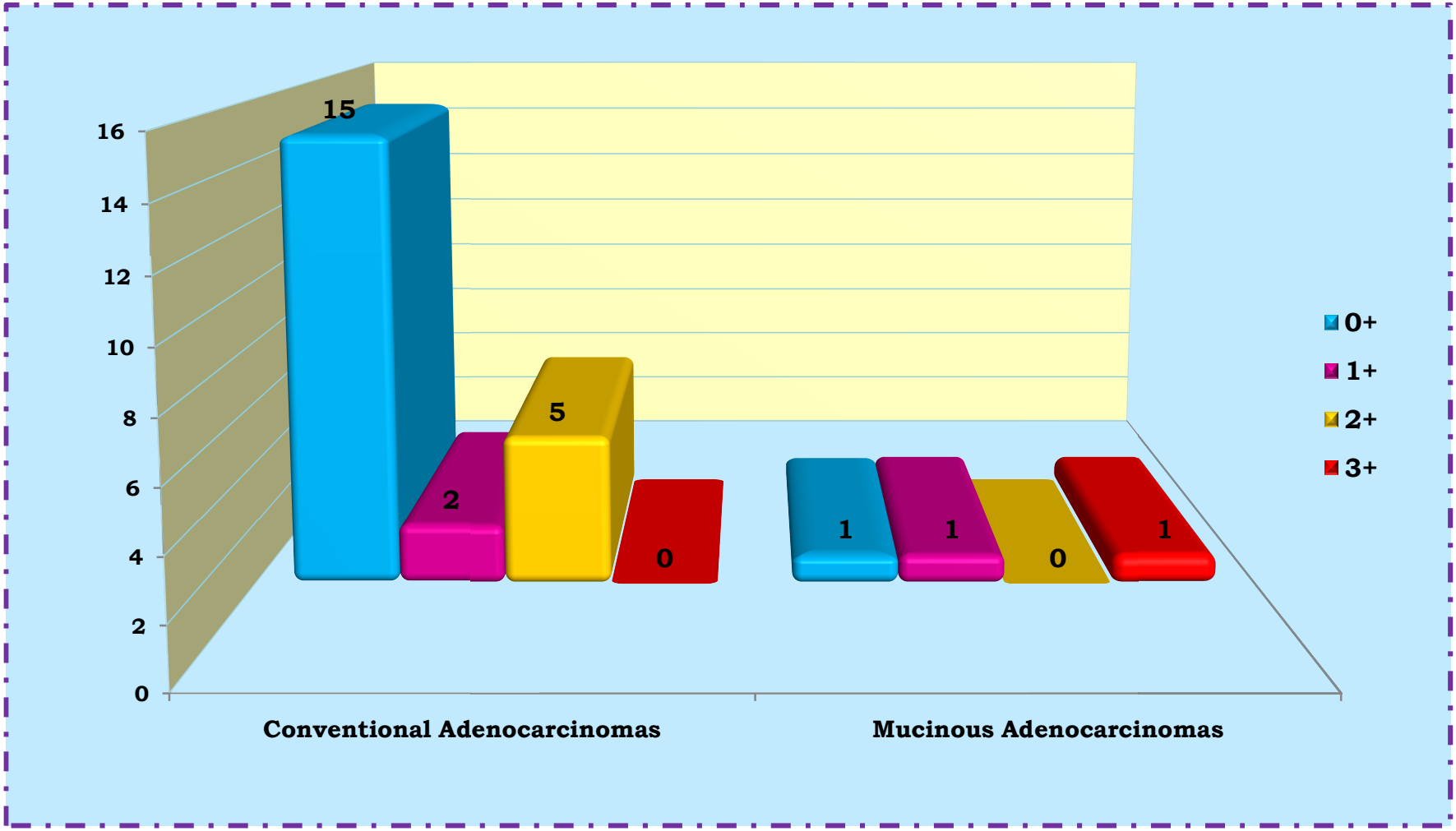


Table - 22

HER2/neu expression in colorectal carcinomas in relation to histological grade

Histological Grade	HER2/neu score				Total
	0	1	2	3	
I	3	1	1	0	5
II	11	1	4	0	16
III	1	0	0	0	1
Total	15	2	5	0	22

From table no.22, among five cases showing moderate membranous positivity (2+) for HER2/neu expression, four cases were grade II tumours (moderately differentiated carcinoma) and one case was grade I tumour (well differentiated carcinoma). p-value for this finding was 0.414 which was not statistically significant.

CHART - 21

HER2/neu expression in colorectal carcinomas in relation to histological grade

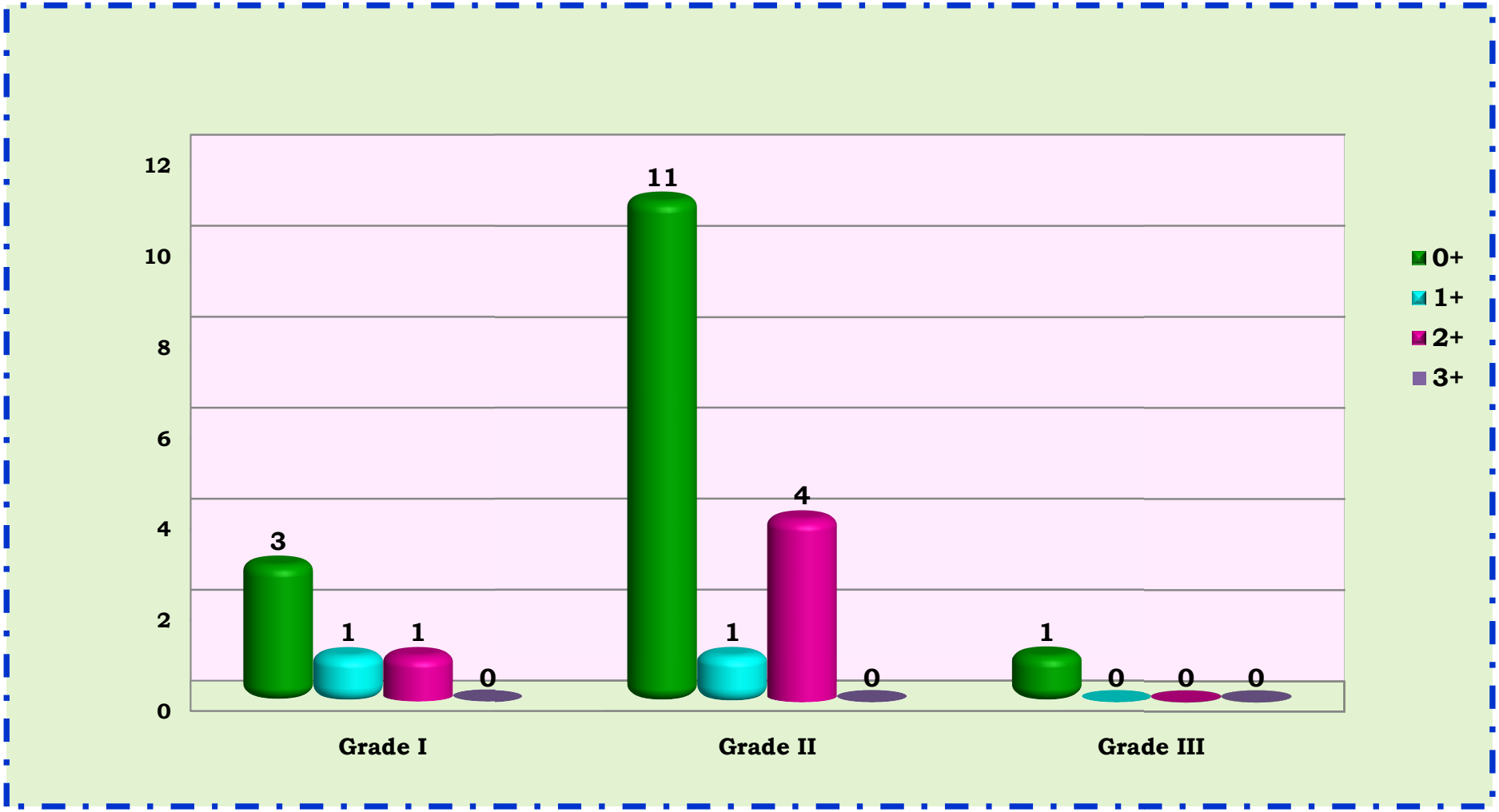


Table - 23

HER2/neu expression in colorectal carcinoma in comparison with tumour stage

Stage (AJCC)	HER2/neu Score				Total
	0	**1**	**2**	**3**	
I	2	1	1	0	4
II	6	0	1	0	7
III	6	2	3	1	12
IV	2	0	0	0	2
Total	16	3	5	1	25

From table no.23, it was evident that strong membranous positivity (3+) was observed in one case of stage III tumours and moderate membranous positivity (2+) was observed in three cases of stage III tumours and one in each of stage I and stage II tumours.

CHART - 22

HER2/neu expression in colorectal carcinoma in comparison with tumour stage

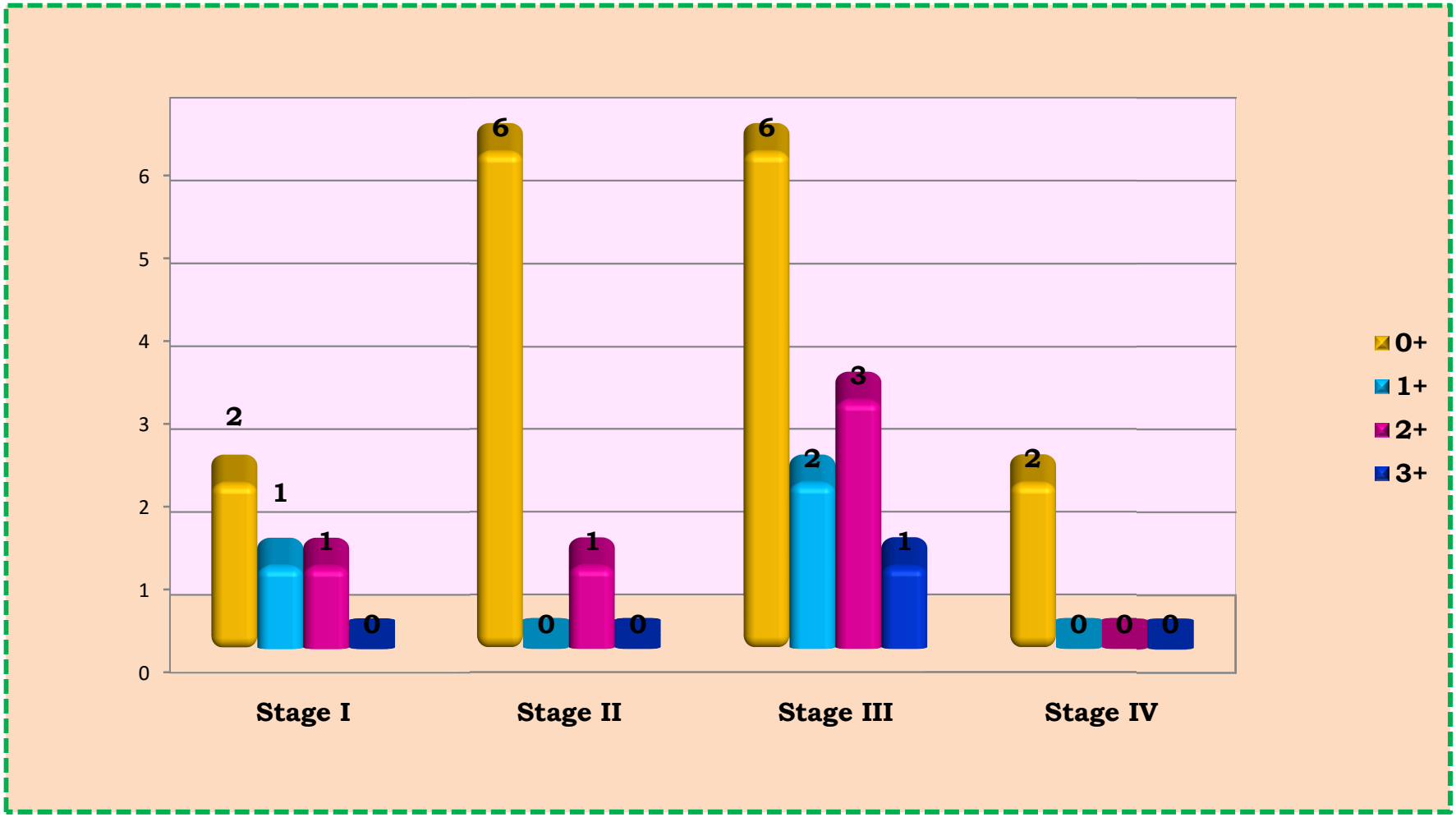


Table - 24

Correlation of HER2/neu expression in colorectal carcinomas with site of tumour

Site	HER2/neu score				Total
	0	**1**	**2**	**3**	
Caecum	1	0	1	1	3
Ascending Colon	4	0	0	0	4
Hepatic Flexure	1	0	0	0	1
Transverse Colon	0	1	0	0	1
Descending Colon	0	0	0	0	0
Sigmoid Colon	4	2	1	0	7
Rectum	6	0	3	0	9
Total	16	3	5	1	25

p-value was 0.025 which was statistically significant.

PHOTOGRAPHS

FIGURE -1

ULCEROPROLIFERATIVE GROWTH IN TRANSVERSE COLON

1342/17 70 Yrs Male

FIGURE – 2

POLYPOIDAL GROWTH IN SIGMOID COLON

587/17 70 Yrs Female

FIGURE – 3

WELL DIFFERENTIATED ADENOCARCINOMA (GRADE- I) SHOWING WELL FORMED GLANDS WITH BASALLY ORIENTED NUCLEI AND WITHOUT SIGNIFICANT STRATIFICATION (100X)

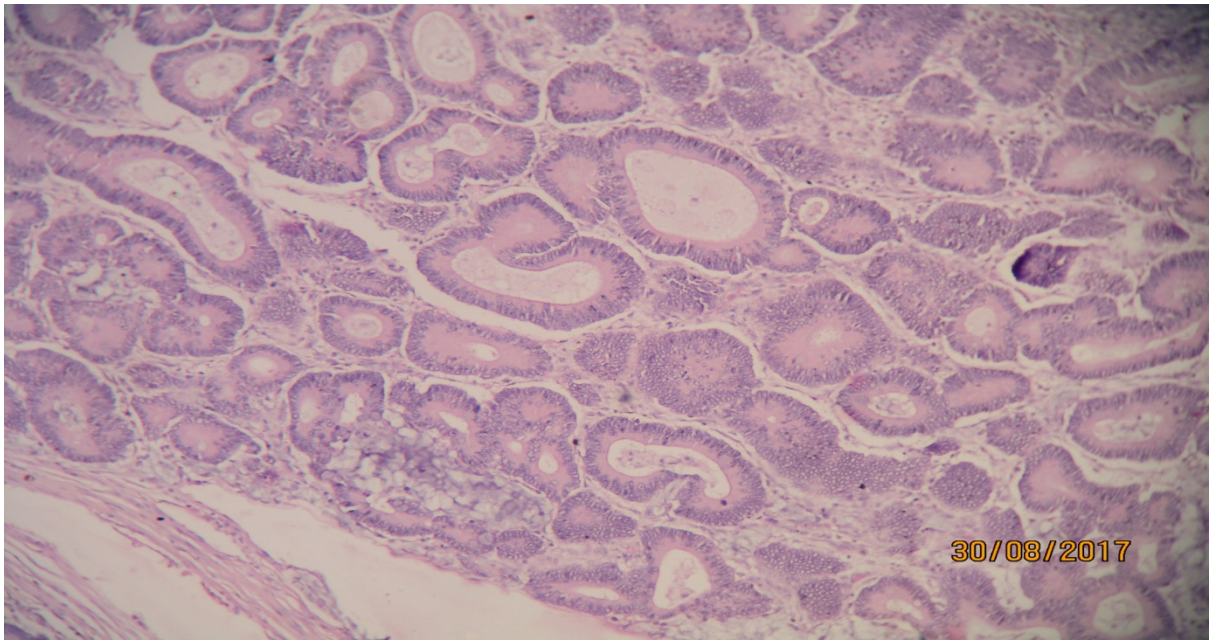


FIGURE – 4

MODERATELY DIFFERENTIATED ADENOCARCINOMA (GRADE -II) SHOWING COMPLEX AND IRREGULAR TUBULAR PATTERN. NUCLEI ARE HIGHLY ATYPICAL, ELONGATED AND STRATIFIED (100X)

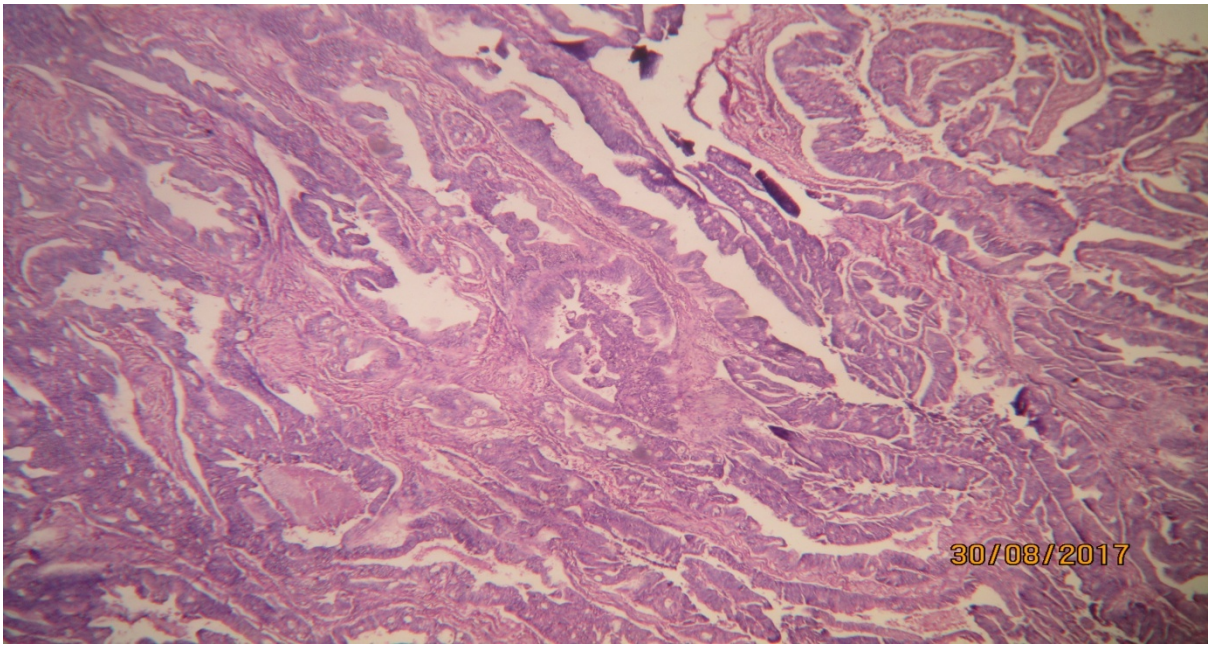


FIGURE – 5

POORLY DIFFERENTIATED CARCINOMA (GRADE – III) SHOWING SOLID SHEETS OF MALIGNANT CELLS WITHOUT GLANDULAR DIFFERENTIATION (100X)

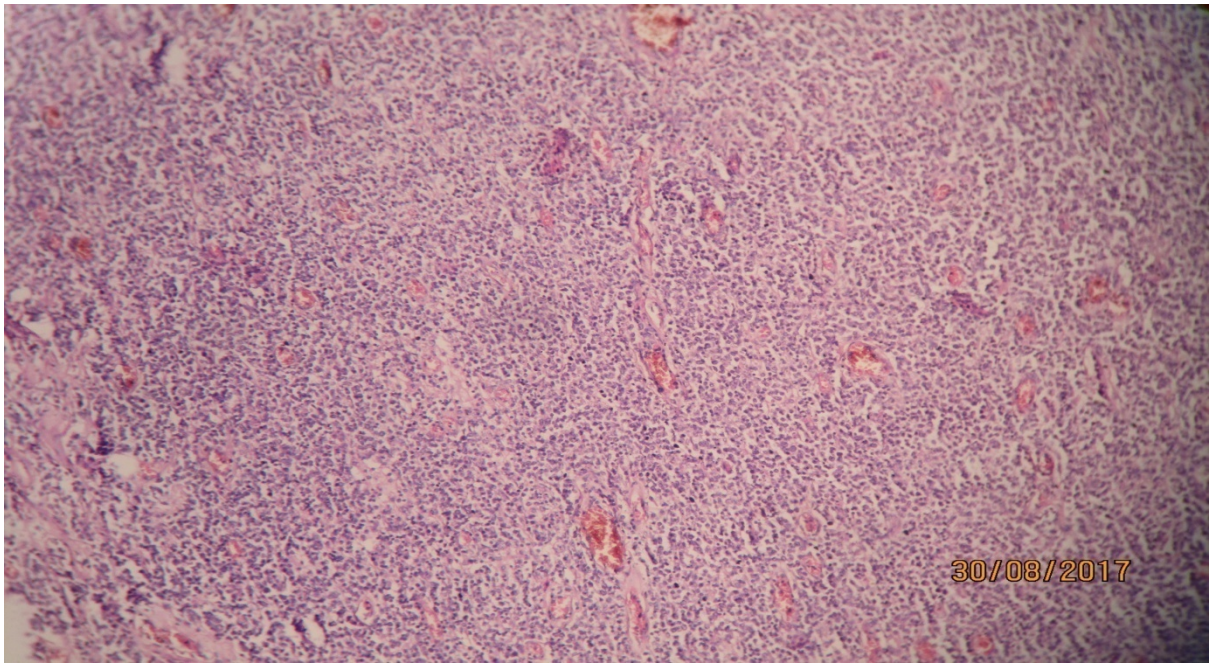


FIGURE – 6

MUCINOUS CARCINOMA SHOWING TUMOUR CELLS FLOATING IN MUCIN POOLS (100X)

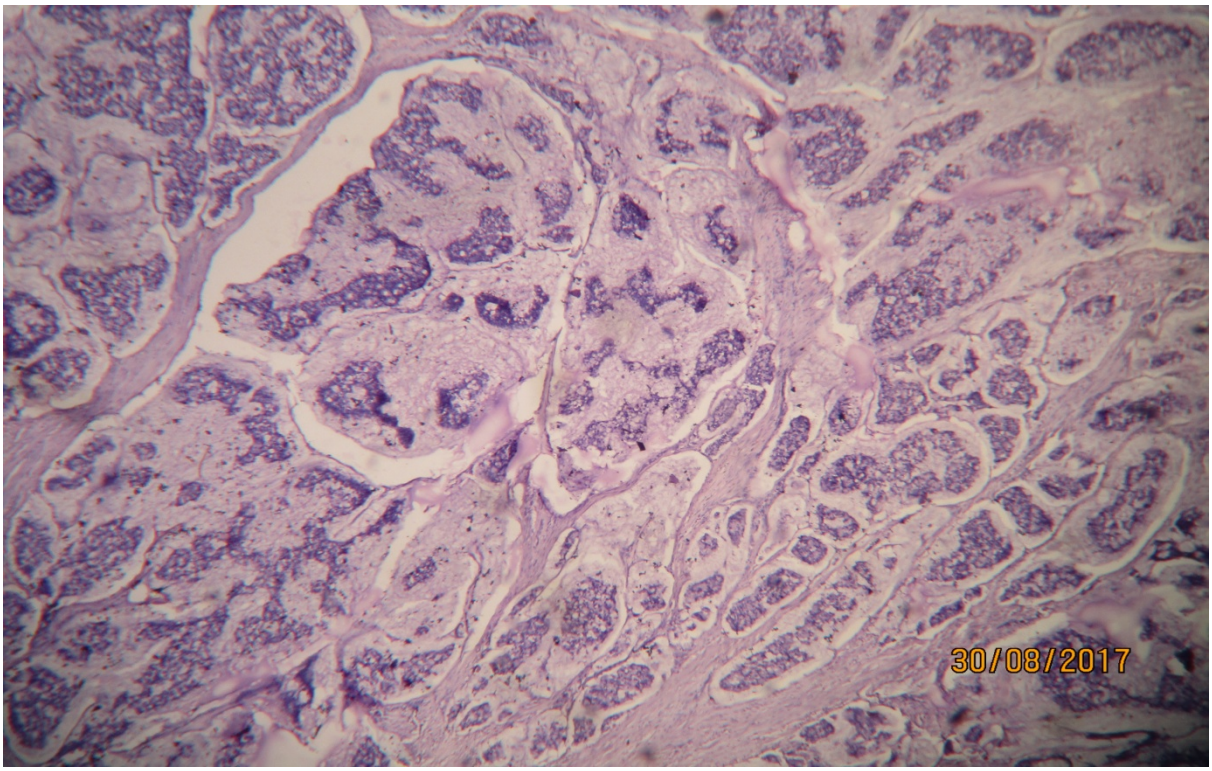


FIGURE – 7

HER2/neu IMMUNOHISTOCHEMISTRY – HER2/neu SCORE 0+ (100X)

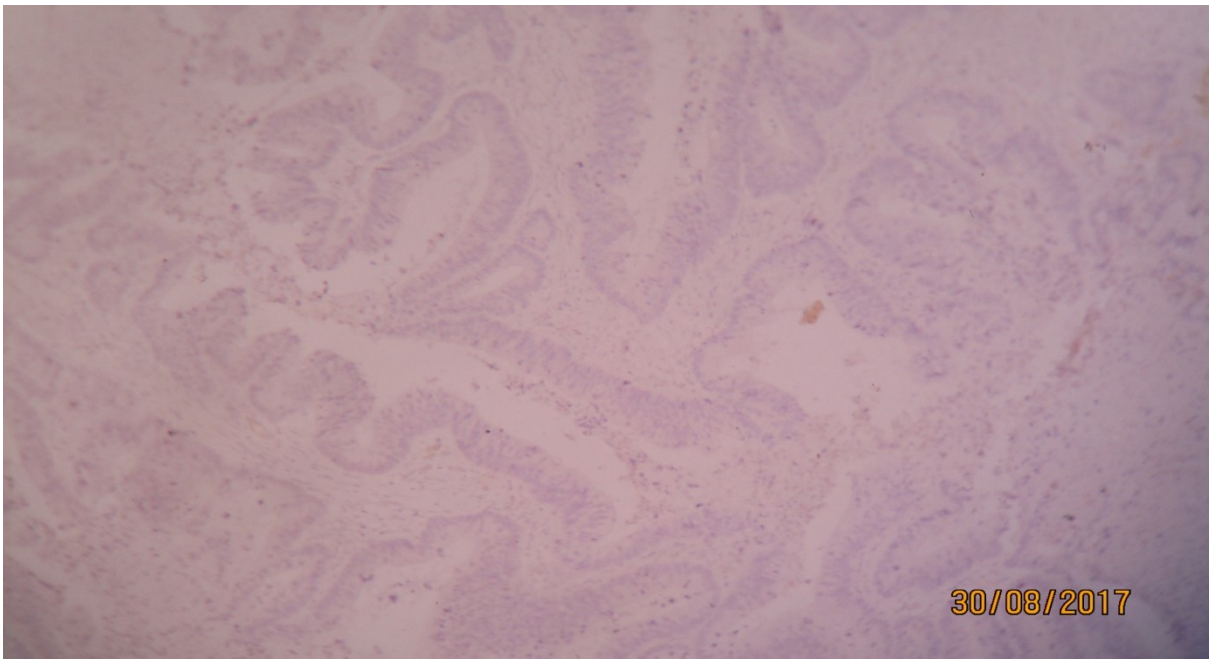


FIGURE – 8

HER2/neu IMMUNOHISTOCHEMISTRY – HER2/neu SCORE 1+ (100X)

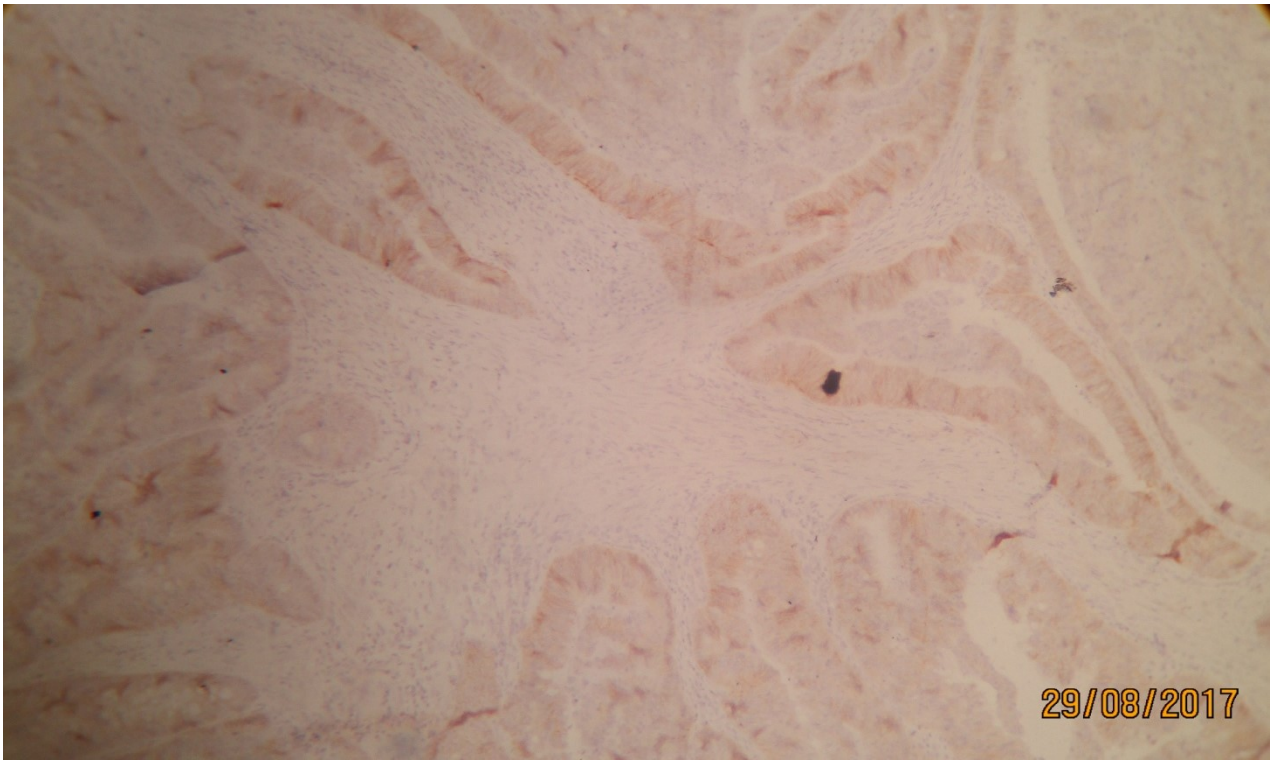


FIGURE – 9

HER2/neu IMMUNOHISTOCHEMISTRY – HER2/neu SCORE 2+ (100X)

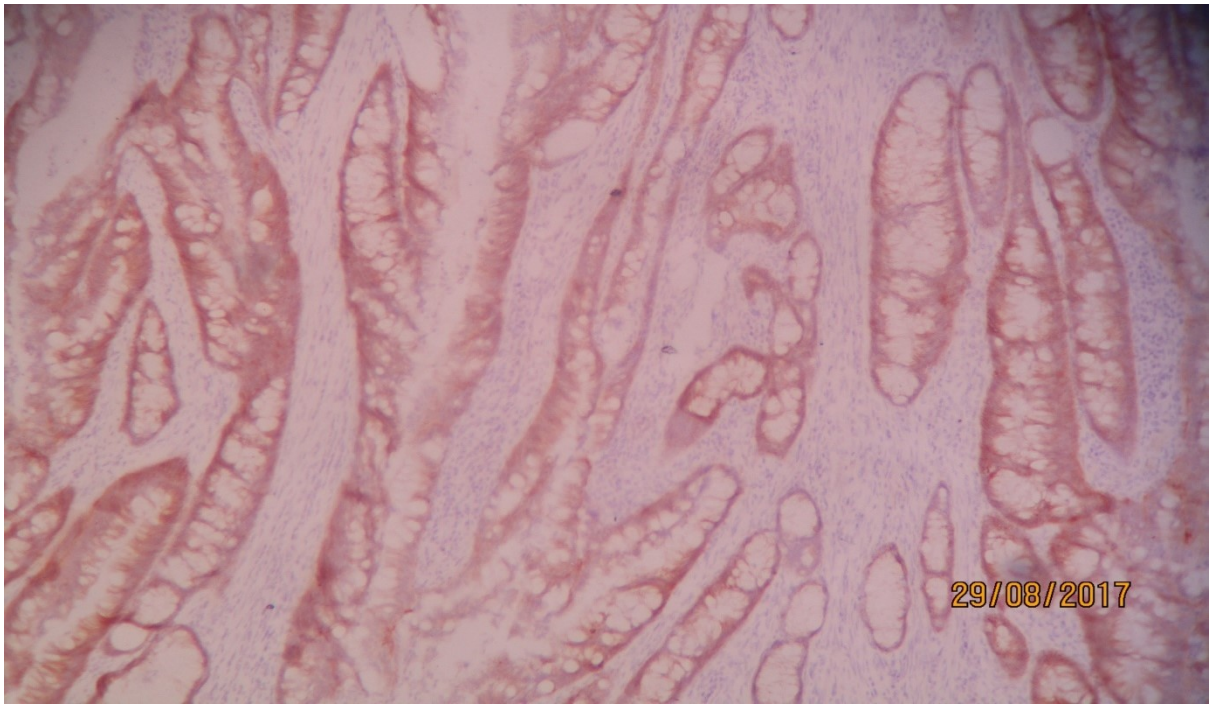
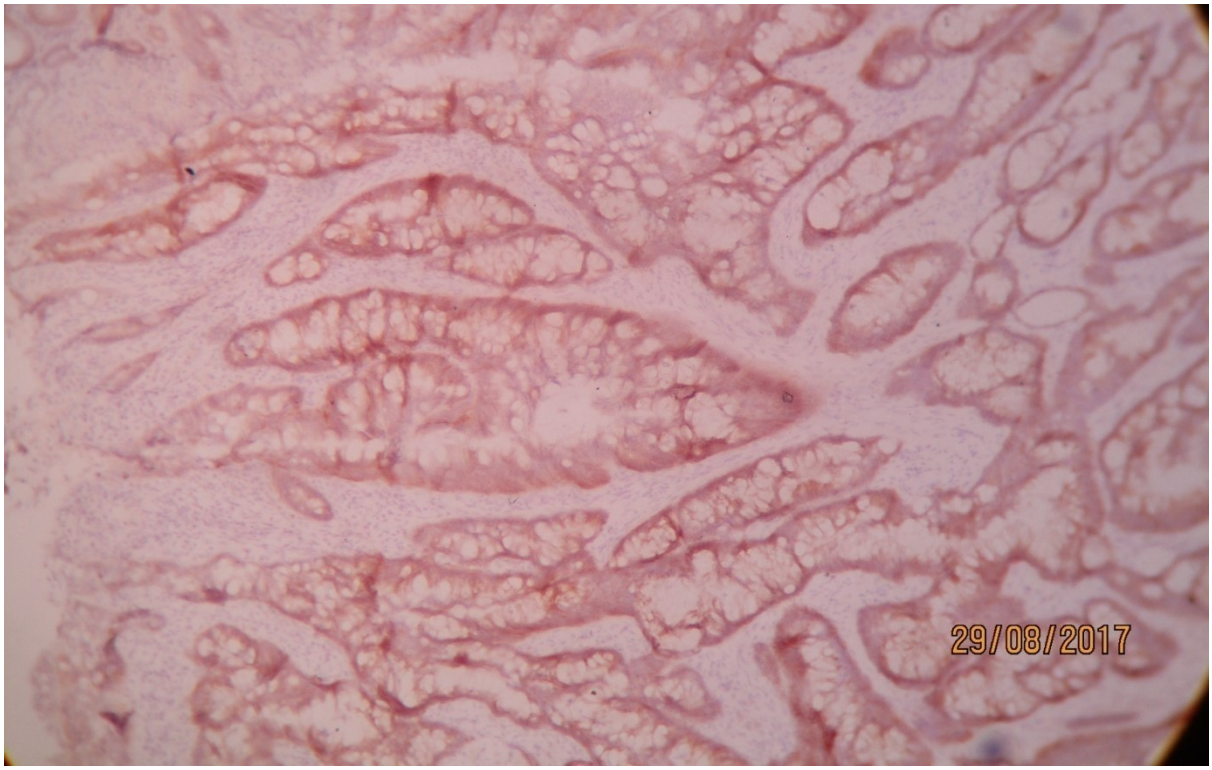


FIGURE – 10

HER2/neu IMMUNOHISTOCHEMISTRY – HER2/neu SCORE 3+ (100X)



DISCUSSION

DISCUSSION

Colorectal carcinoma is one of the most common cancers. Inspite of the improvement in treatment modalities, colorectal carcinoma remains as a leading cause of cancer mortality.

Since the early 1990s, many research activities have been carried out on colorectal cancer. As a result of these researches, the basics of tumour biology and colorectal carcinoma pathogenesis have been understood. In most of the individuals with colorectal carcinoma, cancer development is mainly due to complex interaction between the genetic factors and environmental factors[(2)].

About 85% of tumours of large intestine are adenocarcinomas. The incidence of colorectal carcinoma increases after 50 years of age with peak incidence between 60 and 70 years of age [(4)]. In this study, age group of patients with colorectal carcinoma ranges from 35 to 75 years and peak incidence was found between 40 to 70 years of age.

In this study, 50 colectomy specimens received in Department of Pathology, Thanjavur Medical College, during January 2016 to June 2017 was examined. These specimen were obtained from Department of General Surgery and Surgical Gastroentrology, Thanjavur Medical College. Adequate clinical details were collected for all the cases. Among them, 44 cases were conventional adenocarcinomas and 6 cases were mucinous carcinomas.

Table - 25

S.No	Studies	Age Range (Yrs)
1	Soo Kyung nam et al [21]	28 - 93
2	Won-Suk Lee et al [29]	41 - 87
3	Manmeet Kaur Gill et al [8]	19 - 88
4	Ingold heppner et al [24]	16 - 98
5	Sadia Anwar et al [6]	35 - 80
6	An Na Seo et al [23]	20 - 95
7	JinhuaTu et al [26]	17 - 85
8	Present Study	35 - 75

Table - 26

S.No	Studies	Mean Age (Yrs)
1	Soo Kyung nam et al [21]	60
2	Won-Suk Lee et al [29]	60.8
3	Manmeet Kaur Gill et al [8]	53.9
4	Ingold heppner et al [24]	71
5	Sadia Anwar et al [6]	57.5
6	An Na Seo et al [23]	65
7	JinhuaTu et al [26]	51
8	Present Study	55.8

In the present study, age of colorectal carcinoma patients ranges from 35 - 75 years and mean age is 55.8 years. This was in concordance with the study done by Sadia Anwar et al [6]and Manmeet Kaur Gill et al[8] (Table .25& 26)

Table - 27

S.No	Studies	Total Cases	Male	Female
1	JinhuaTu et al [26]	878	541	337
2	An Na Seo et al [23]	539	296	243
3	Ingold Heppner et al [24]	1645	839	806
4	Manmeet Kaur Gill et al [8]	40	24	16
5	Won-Suk Lee et al [29]	94	66	28
6	Soo Kyung Nam et al [21]	191	103	88
7	Pappas et al[5]	51	18	33
8	Present Study	50	27	23

In the present study, out of 50 colorectal carcinoma patients, 27 cases were males and 23 cases were females. Thus, in this study, male preponderance was seen among colorectal carcinoma patients. This was in accordance with the study done by JinhuaTu et al [26],An Na Seo et al [23], Ingold Heppner et al [24], Manmeet Kaur Gill et al [8], Won - Suk Lee et al [29] and Soo Kyung Nam et al [21] (Table No.27).

Most of the proximal colonic carcinomas remain asymptomatic and usually presents at an older age group whereas common complaints of distal

colorectal carcinomas were change in bowel habits, abdominal pain and bleeding per rectum[2].In this study also, altered bowel habits was the chief complaint noticed in 70% of patients followed by abdominal pain in 40% of patients (Table No.2).

Among the tumours of colorectal region, rectal carcinomas are commonly encountered in Asians [4]. In this study also, the commonest site affected by colorectal carcinoma was rectum followed by sigmoid colon and caecum. This was encountered in all age groups of patients.

Table- 28

Location of colorectal carcinoma in different studies

(expressed in percentage)

S.No	Site	Abdul Kareem et al [46] (%)	Osime et al [47] (%)	Qizilbash et al [48] (%)	Present study (%)
1	Caecum	9	2.63	13.53	16
2	Ascending Colon	6	2.63	11.28	12
3	Hepatic Flexure	0	1.32	1.50	4
4	Transverse Colon	4.5	1.32	4.89	2
5	Splenic Flexure	0	1.32	2.63	0
6	Descending Colon	3.6	3.94	6.77	0
7	Sigmoid Colon	0	7.89	34.21	16
8	Recto Sigmoid	58.8	0	9.02	0
9	Rectum	0	78.95	14.28	50
`	Total no of cases	420	76	266	50

In the present study, out of 50 colorectal carcinomas, 33 were situated in the distal colorectal region (sigmoid colon and rectum). Among them, 25 cases

of colorectal carcinoma were situated in rectum which was the commonest site affected (50%). This was in accordance with the study done by Osime U et al [47] (Table No.28)

The proximal colonic tumours usually present as ulceroproliferative growth and distal colorectal tumours commonly present as annular lesion resulting in narrowing of lumen[4].

Table - 29

Growth Patterns of Colorectal Carcinomas

S.No	Studies	Ulceroproliferative (%)	Polyp (%)	Narrowing of Lumen(%)
1	Qizilbash et al [48]	54	28	18
2	Present Study	74	6	20

Ulceroproliferative growth was the commonest colonoscopic findings found in colorectal carcinoma [2]. In the present study, 74% of tumours presented as ulceroproliferative growth. This includes both right sided and left sided colonic tumours (Table No.3). This was in accordance with the study done by Qizilbash et al [48](Table No.29). There was a correlation between HER2/neu expression and configuration which was statistically significant(p=0.013).

Table - 30

Studies relating sizes of colorectal carcinoma

S.No	Studies	Upto 5Cm	More than 5Cm	Total
1	JinhuaTu et al [26]	445	433	878
2	Present Study	32	18	50

In this present study, out of 50 cases of colorectal carcinoma, 32 cases had tumour size less than 5cm size (Table No.6). This correlates with the study by JinhuaTu et al [26] (Table No.30). Since nowadays, many medical camps and screening facilities are available for the patients, their earlier detection of colorectal carcinoma may be the reason behind this less tumour size i.e.less than 5cm size.

Table - 31

Incidence of mucinous and non-mucinous adenocarcinoma

S.No	Studies	Total no. of cases in study	Mucinous carcinoma		Non-mucinous adenocarcinoma	
			No. of cases	%	No. of cases	%
1	Abdul Kareem et al [46]	405	45	10.7	360	89.3
2	Osime et al [47]	76	4	5.2	69	90.7
3	Qizilbash et al [48]	266	14	5	244	91.7
4	Fazeli et al [49]	403	71	17.6	332	82.4
5	Present study	50	6	12	44	88

Mucinous carcinomas constitutes about 10-15% of adenocarcinomas of colorectal region. About 85% of tumours of colorectal region were conventional adenocarcinomas[2]. In this present study, about 12% of tumours were mucinous adenocarcinomas and remaining 88% of tumours are conventional adenocarcinomas. This was similar to the findings of Abdul Kareem et al [46] (Table No.31).

Most of the mucinous carcinoma present at advanced stage of disease [15]. In present study, out of 6 cases of mucinous carcinoma, 4 cases presented at stage III disease and one case presented at stage II disease. The 5 year survival rate of mucinous carcinomas are estimated to be 43.1% whereas 5 year survival rate of conventional adenocarcinomas was estimated to be 79.4% when presented at stage II and stage III[50].

Table - 32

Comparison of histological grades of adenocarcinoma

S.No	Studies	Grade I		Grade II		Grade III		Total
		No.of cases	%	No.of Cases	%	No.of cases	%	
1	Pappas et al [7]	4	7.8	43	84.3	4	7.8	51
2	Ingold Heppner et al [24]	46	2.9	1253	78.5	298	18.7	1597
3	Won - Suk Lee et al [29]	37	39.4	53	56.4	4	4.2	94
4	Manmeet Kaur Gill et al [8]	16	40	13	32.5	2	5	40
5	Present study	10	20	33	66	1	2	50

In this study, 33 cases of 50 colorectal carcinoma are moderately differentiated carcinomas (Grade II tumours). This correlates with the study done by Pappas et al [7], Ingold Heppner et al [24], Won-Suk Lee et al [29] (Table No.32). In the present study, Grade II tumours are most common followed by Grade I tumours (well differentiated carcinomas).

Table - 33

Comparison of TNM staging of adenocarcinoma in various studies

S.No	Studies	Stage I		Stage II		Stage III		Stage IV		Total
		No.of cases	%	No.of Cases	%	No.of cases	%	No of cases	%	
1	Ingold Heppner et al[24]	346	21.0	546	33.2	567	34.5	186	11.3	1645
2	JinhuaTu et al [26]	12	1.36	174	19.8	648	73.8	44	5.01	878
3	An Na Seo et al [23]	46	12.6	118	32.3	135	37.0	66	18.1	365
4	Fazeli et al [44]	33	8.2	193	48.1	134	33.04	41	10.2	401
5	Present study	17	34	12	24	18	36	3	6	50

In this study, 18 cases of 50 colorectal carcinomas presented at stage III which was more frequent. This was similar to the results of Ingold Heppner et al [24], JinhuaTu et al [26] and An Na Seo et al [23]. (Table No.33).

HER2/neu expression in colorectal carcinomas

In the present study, 25 cases of 50 colorectal carcinoma cases were selected for HER2/neu immunohistochemistry.

Table - 34

S.No	Studies	HER2/neu expression (%)
1.	Pappas et al [5]	3.9
2	Nathanson et al [30]	3.6
3	Kavanagh et al [31]	10
4	Park et al [32]	47.4
5	Schuell et al [34]	4
6	JinhuaTu et al [26]	11.6
7	Li et al [42]	15.5
8	Lazaris et al [43]	15.5
9	Kim et al [38]	0.5
10	Present study	12

In this study, 12% of colorectal carcinoma cases showed HER2/neu overexpression. Out of them, 2% (n=1) cases showed strong membranous positivity (3+) and 10% (n=5) cases showed moderate membranous positivity (2+) for HER2/neu immunohistochemistry. The percentage of HER2/neu expression varied in different studies (Table No.34). This may be due to

interobserver variability and different scoring system used. In some studies, cytoplasmic staining were also considered as positive.

In the present study moderate membranous staining (2+) HER2 expression was detected in five conventional adenocarcinomas and strong membranous staining (3+) was detected in one mucinous carcinoma (Table no.20). There was no association between HER2/neu expression and histological variant of colorectal carcinoma (p=0.114). This was in accordance to the study done by Marx et al [39] and Kruszewski et al [37].

In this study, out of 5 cases showing 2+ HER2 expression, four cases belongs to moderately differentiated tumours (Grade II) and one case belongs to well differentiated tumour (Grade I). 3+ HER2 staining was detected in one mucinous carcinoma (Table No.21). But, there was no correlation between HER2/neu expression and tumour grade (p=0.414). This was not statistically significant. This was similar to the results of Kavanagh et al [31], JinhuaTu et al [26], Kruszewski et al [37], Marx et al [39].

In our study, 3+ membranous immunostaining was noticed in one stage III tumour and 2+ membranous immunostaining was noticed in three stage III tumours and one in each stage I and stage II tumour (Table No.23). But, this association was not statistically significant (p=0.228). There was no relationship between HER2/neu expression and tumour stage. This was similar to the results

of Sadia Anwar et al [6], Kavanagh et al [31], JinhuaTu et al [26], Kruszuoski et al [37].

In this study, one of the proximal colonic tumour (caecum) expressed strong membranous immunostaining whereas moderate membranous immunostaining was expressed in one proximal colonic tumour and four distal colorectal tumours. This association was found to be statistically significant (p=0.025). This was in accordance with the study done by An Na Seo et al [23].

There was no correlation between HER2 expression and clinicopathological parameters like age, sex, clinical presentation, tumour size, tumour histology, tumour grade and stage .

There was statistically significant association between HER2/neu expression and tumour site. Also, there was correlation between HER2/neu expression and tumour configuration.

Many breast cancer patients have been benifited after the development of monoclonal antibody therapy against HER2/neu. This leads to evaluation of HER2/neu expression in other tumour types also, since it can be used as a therapeutic target. HER2 targeted therapy have been used in metastatic gastric cancer and this leads to 37% improvement in survival rate. This leads to approval of trastuzumab by United States Food and Drug Administration for patients having HER2 positive metastatic lesions [29].

Since HER2/neu expression correlates with poorer prognosis, monoclonal antibody therapy like trastuzumab (herceptin) can be used in colorectal carcinoma patients with HER2/neu overexpresssion.

SUMMARY

&

CONCLUSION

SUMMARY AND CONCLUSION

- This was a longitudinal retrospective study carried out in our Department of Pathology, Thanjavur Medical College during period of January 2016 to June 2017.
- Only resected colectomy specimens which was diagnosed as colorectal carcinoma was included in this study.
- Total number of colorectal carcinoma in the study - 50.
- All these cases had adequate clinical details and colonoscopic findings were noted.
- Histopathological examination was done to asses the grade and tumour invasion.
- TNM staging was used to assess the stage of colorectal carcinomas.
- HER2/neu overexpression was evaluated in 25 out of 50 cases using immunohistochemistry.
- Age group of colorectal carcinoma patients ranges from 35 to 75 years of age with mean age of 55.8 years.
- Males are predominantly affected than females.
- The common presenting complaints was altered bowel habits (70%) followed by abdominal pain (40%).
- 74% of colorectal carcinoma present grossly as ulceroproliferative growth.
- Rectum was the commonest site affected followed by sigmoid colon and caecum.

- Conventional adenocarcinoma constitutes 88% of colorectal carcinomas whereas mucinous carcinoma constitutes 12%.
- Grade II tumours (moderately differentiated carcinomas) constitutes 66% of conventional adenocarcinomas.
- Most of the colorectal carcinomas presented at stage III (36%).
- Low grade tumours were commonly seen in males whereas high grade tumours were commonly seen in females.

HER2/neu OVEREXPRESSION

- HER2/neu overexpression was noticed in 12% of colorectal carcinoma cases which includes moderate membranous staining (2+) in 10% of cases and strong membranous staining (3+) in 2% of cases.
- There was a statistically significant correlation between HER2/neu expression with tumour site and configuration.
- No association was found between HER2/neu expression and other clinicopathological variables. This includes age, gender, clinical presentation, tumour grade, histological variant and staging.
- Anti-epidermal growth factor receptor monoclonal antibody therapy like trastuzumab can be used in these HER2/neu overexpressed colorectal carcinoma patients since they are potential candidates.

APPENDIX

APPENDIX I

HAEMATOXYLIN AND EOSIN STAIN

Preparation of solution:

HARRIS HAEMATOXYLIN

Distilled water -1000ml

Ammonium alum -100gm

Absolute ethyl alcohol -50ml

Mercuric oxide -2.5gm

100g of ammonium alum dissolved in 1000ml of distilled water by heating and shaking at 60°c. Add solution of 5 g of haematoxylin in 50 ml of ethylalcohol and bring rapidly to boil. When it begins to boil, remove from flame and add 2.5g of mercuric oxide. Mix by swirling gently.

EOSIN STAIN

Eosin Y -1gm

Distilled water -20ml

95% ethanol -80ml

Glacial acetic acid -0.2ml

Dissolve 1 gm of eosin Y in 20ml of water,add 80 ml of 95 % ethyl alcohol and 0.2 ml of glacial acetic acid.

Procedure:

1. Bring the sections to water.
2. Dip in Harris haematoxylin for 15 minutes.
3. Rinse in tap water.
4. Differentiate in 1% acid alcohol, 3-4 quick dips.
5. Wash it in tap water briefly.
6. Dip in ammonia water or saturated lithium carbonate until the sections are blue.
7. Wash in running tap water for 10-20 minutes.
8. Stain with eosin for 15 minutes depending on the age of eosin and the depth of counter stain.
9. Rinse in tap water.
10. Dip in 95% alcohol.
11. 3 changes in absolute alcohol.
12. Xylene – 2 changes.
13. Mount in DPX mountant.

APPENDIX II

IMMUNOHISTOCHEMISTRY

PREPERATION OF SOLUTIONS

Tris Buffer Saline (TBS) -0.005M

Distilled water -10 litres

Sodium chloride -80 gm

Tris (hydroxymethylamine) -6.05gm

1 N Hydrochloric acid -44ml

Final pH is adjusted to 7.6 with either 1N Hcl or 0.2 M Tris solution

CITRATE BUFFER SOLUTION

Trisodium citrate -2.94gm

1N Hydrochloric acid -5 ml

Distilled water -1000ml

Final pH is adjusted to 6.0 with 1N Hcl

Preparation of gelatine coated slides:

Chrome alum -0.05gm

Gelatin -0.3 gm

Distilled water -100 ml

Chrome alum is added to distilled water and then heated to 60°c,gelatine is added slowly to heated distilled water. Glass slides are then dipped in this solution dried overnight.

Antigen retrieval:

The slides are placed in citrate buffer in the coplin jar and capped. The jar is then heated in a 750w domestic microwave oven for 15 minutes.

Procedure:

1. Dewax the section in xylene (1/2 hr, 2 changes) and bring section to distilled water.
2. Antigen retrievel using TBS by microwave oven heating.
3. Cool to room temperature in running tap water for 20 minutes.
4. Bring the section to TBS for 5 minutes.
5. Drain and wipe of excess TBS around sections.
6. Incubate in endogenous peroxidase blocking agent for 15-20 minutes.
7. Gently wash the slides in TBS for 5 minutes.
8. Wipe off excess fluid and incubate in power block for 15-20 minutes.
9. Blot and dry excess power block.
10. Incubate in primary antibody for 60 minutes.
11. Repeat steps 4 and 5.
12. Incubate in super enhancer for 30 minutes.
13. Repeat steps 4 and 5.

14. Incubate in secondary antibody for 60 minutes.
15. Repeat steps 4 and 5.
16. Incubate in DAB (DiaminoBenzidine) substrate buffer for 2 -10 minutes.
17. Wash in distilled water, counter stain with haematoxylin,clear in xylene and mount with DPX.

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MASTER CHART

S.No	HPE. No.	Age	Sex	BPR	ALT BH	LOW	Site	Size	Configuration	Histology	Grade	Stage	HER2/neu
1	14/16	42	M	Y	N	N	RECTUM	Up to 5CM	POLYP	MULTIPLE POLYPOSIS COLON	I	III	2
2	70/16	43	M	N	N	N	RECTUM	Up to 5CM	ULCERO PROLIFERATIVE	MUCIN SECRETING ADENOCA	I	III	
3	202/16	65	F	N	Y	N	RECTUM	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	I	
4	451/16	53	F	N	Y	N	ASCENDING COLON	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	III	II	0
5	553/16	70	F	N	Y	Y	HEPATIC FLEXURE	Up to 5CM	ULCERO PROLIFERATIVE	MUCINOUS CARCINOMA		I	
6	946/16	60	F	N	Y	N	CAECUM	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	II	
7	1153/16	50	M	N	Y	N	SIGMOID COLON	Up to 5CM	NAROWING OF LUMEN	ADENO CA	II	III	0
8	1264/16	53	M	Y	N	Y	RECTUM	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	I	
9	1321/16	35	M	N	Y	N	CAECUM	6-10 CM	ULCERO PROLIFERATIVE	ADENO CA	II	I	
10	1331/16	45	F	N	Y	N	SIGMOID COLON	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	II	
11	1567/16	73	M	Y	N	N	RECTUM	6-10 CM	ULCERO PROLIFERATIVE	ADENO CA	II	I	0
12	1571/16	70	F	N	N	N	RECTUM	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	I	II	0
13	1624/16	75	M	N	Y	N	ASCENDING COLON	6-10 CM	ULCERO PROLIFERATIVE	ADENO CA	II	III	
14	1710/16	53	M	N	Y	Y	CAECUM	Up to 5CM	ULCERO PROLIFERATIVE	MUCIN SECRETING ADENOCA	I	III	
15	1754/16	37	M	Y	N	N	RECTUM	6-10 CM	ULCERO PROLIFERATIVE	ADENO CA	II	I	
16	1799/16	55	F	N	Y	N	SIGMOID COLON	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	IV	0
17	1902/16	50	F	Y	N	N	RECTUM	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	I	
18	2251/16	45	F	Y	N	N	RECTUM	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	III	
19	2368/16	58	M	N	Y	Y	RECTUM	>10 CM	ULCERO PROLIFERATIVE	ADENO CA	II	II	0

S.No	HPE. No.	Age	Sex	BPR	ALT BH	LOW	Site	Size	Configuration	Histology	Grade	Stage	HER2/neu
20	2470/16	50	F	Y	Y	N	RECTUM	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	II	
21	2624/16	54	M	N	Y	N	SIGMOID COLON	Up to 5CM	POLYP	ADENO CA	II	III	1
22	2671/16	40	F	Y	Y	N	RECTUM	6-10 CM	ULCERO PROLIFERATIVE	ADENO CA	I	III	0
23	3077/16	53	F	Y	N	N	RECTUM	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	I	I	
24	3154/16	75	M	N	Y	N	RECTUM	Up to 5CM	NARROWING OF LUMEN	ADENO CA	II	II	2
25	3236/16	50	F	Y	N	N	RECTUM	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	IV	
26	3425/16	60	M	N	Y	Y	ASCENDING COLON	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	II	
27	3947/16	60	F	N	Y	N	SIGMOID COLON	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	III	2
28	3969/16	35	F	Y	Y	N	RECTUM	6-10 CM	ULCERO PROLIFERATIVE	MUCINOUS CA		II	
29	4081/16	53	M	N	N	N	CAECUM	6-10 CM	ULCERO PROLIFERATIVE	MUCIN SECRETING ADENO CA	I	I	
30	4092/16	70	M	Y	Y	N	RECTUM	6-10 CM	ULCERO PROLIFERATIVE	ADENO CA	II	I	
31	4131/16	75	M	N	Y	N	HEPATIC FLEXURE GROWTH	>10 CM	ULCERO PROLIFERATIVE	ADENO CA	II	II	0
32	4147/16	55	M	N	Y	Y	SIGMOID COLON	6 -10 CM	ULCERO PROLIFERATIVE	ADENO CA	I	I	1
33	4314/16	40	F	N	Y	N	RECTUM	Up to 5CM	NARROWING OF LUMEN	MUCIN SECRETING ADENO CA	I	I	
34	4320/16	50	F	Y	N	N	RECTUM	6-10 CM	ULCERO PROLIFERATIVE	ADENO CA	II	I	
35	4719/16	60	F	N	Y	N	ASCENDING COLON	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	I	0
36	115/17	40	F	N	Y	N	CAECUM	Up to 5CM	ULCERO PROLIFERATIVE	MUCINOUS CA		III	3
37	230/17	61	M	N	N	Y	CAECUM	>10 CM	ULCERO PROLIFERATIVE	MUCINOUS CA		III	

S.No	HPE. No.	Age	Sex	BPR	ALT BH	LOW	Site	Size	Configuration	Histology	Grade	Stage	HER2/neu
38	289/17	66	M	N	Y	N	ASCENDING COLON	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	III	0
39	530/17	59	F	N	Y	N	RECTUM	Up to 5CM	NAROWING OF LUMEN	ADENO CA	II	III	2
40	587/17	70	F	N	Y	N	SIGMOID COLON	6 -10 CM	POLYP	MUCINOUS CA		III	0
41	694/17	45	F	N	Y	N	CAECUM	Up to 5CM	NAROWING OF LUMEN	ADENO CA	II	IV	0
42	706/17	36	M	N	Y	N	CAECUM	Up to 5CM	NAROWING OF LUMEN	ADENO CA	II	I	2
43	1059/17	75	M	N	Y	N	SIGMOID COLON	Up to 5CM	NAROWING OF LUMEN	ADENO CA	II	II	0
44	1185/17	64	M	Y	N	N	RECTUM	6-10 CM	ULCERO PROLIFERATIVE	ADENO CA	I	II	0
45	1200/17	46	M	N	Y	N	RECTUM	6-10 CM	NAROWING OF LUMEN	ADENO CA	II	III	
46	1342/17	70	M	N	Y	Y	TRANSVERSE COLON	>10 CM	NAROWING OF LUMEN	MUCINOUS CA		III	1
47	1591/17	74	M	N	Y	N	RECTUM	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	III	0
48	1684/17	47	M	N	Y	N	ASCENDING COLON	6-10 CM	ULCERO PROLIFERATIVE	ADENO CA	II	III	0
49	1736/17	55	M	N	Y	N	RECTUM	Up to 5CM	NAROWING OF LUMEN	ADENO CA	II	I	
50	1984/17	70	F	Y	N	N	RECTUM	Up to 5CM	ULCERO PROLIFERATIVE	ADENO CA	II	I	

BPR - BLEEDING PER RECTUM

ALT BH - ALTERED BOWEL HABITS

LOW - LOSS OF WEIGHT

HPE - HISTOPATHOLOGICAL EXAMINATION